

METABOLIC RESPONSES TO ACUTE AND PROLONGED HYPOXIC EXPOSURE

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ABSTRACT

This thesis examined the metabolic effects of acute and intermediate hypoxic exposure in humans, specifically, physiological mechanisms associated with weight loss. Namely; increased metabolic rate, changes in substrate oxidation, altered lipid metabolism and changes in taste.

Study one assessed the validity and reproducibility of an online gas analyser in normobaric hypoxia [Fraction of inspired oxygen: 0.12 (FiO_2 :0.12) equivalent to approximately 4,500m] (n=nine; two females, seven males). The MetaMax3x demonstrates good reproducibility between repeated trials. Differences exist between the system and the gold standard Douglas Bag method for measures of oxygen uptake (percent differences of $\dot{V}\text{O}_2$; 21%), carbon dioxide production ($\dot{V}\text{CO}_2$; 10%) and minute ventilation (\dot{V}_E ; 5%).

The second study investigated the free fatty acid (FFA) and triglyceride (TAG) response to an acute (45 minutes) hypoxic exposure (FiO_2 : 0.12) (n=10; five females, five males). A greater resting metabolic rate (RMR) ($+28 \pm 6 \text{ kcal}\cdot\text{hr}^{-1}$) was observed, through increased carbohydrate (CHO) and fat oxidation. Increased plasma FFA (+54%) and TAG (+26%) were observed, highlighting metabolic perturbations from acute exposure.

Study three investigated the metabolic responses to an acute (60 minute) hypoxic exposure (FiO_2 : 0.12) at rest and a subsequent bout of moderate exercise in normoxia following a high fat meal (n=eight males). Experimental trials included a lipid ingestion prior to a rest period at hypoxia or normoxia followed by moderate intensity exercise (60% heart rate reserve). Control trials consisted of the same protocol without lipid ingestion. Acute, severe hypoxia increased energy expenditure (EE), ($+22 \pm 11 \text{ kcal}\cdot\text{hr}^{-1}$) CHO and fat oxidation following exposure. A prior acute bout of severe hypoxia did not alter EE and substrate use during subsequent moderate intensity exercise. An exercise bout, post-lipid ingestion, resulted in lower triglyceride concentration. No changes in Meteorin-like were observed throughout trials. These findings suggest that an increase in RMR occurs following a single resting hypoxic exposure and independently to Meteorin-like protein.

The fourth study observed reductions in body mass ($-2.36 \pm 1.41 \text{ kg}$) and increases in CHO oxidation during an altitude stay in Peru (18 days, 3400 m) (n=10; five females, five males). The reduction in body mass ($-1.89 \pm 1.31 \text{ kg}$) was sustained four weeks post-return to sea-level. Salt, sweet and bitter taste sensations were reduced at 3,400 m compared to sea-level. No changes in self-reported appetite were observed throughout the testing period. Furthermore no changes in circulating Meteorin-like protein were observed upon return to sea-level at one and four weeks post-altitude stay.

Study five investigated the blood lipid response to a high lipid meal consumed one and four weeks post-return to sea-level following an altitude stay (18 days, 3400 m) (n=10; five females, five males). No lasting postprandial effects were observed. It is likely that a time dependent effect of hypoxia exists with regards to postprandial blood lipid responses.

Taken together acute and intermediate exposure to hypoxic conditions alter substrate oxidation with the potential to induce losses in body mass, independently to changes in Meteorin-like protein and self-reported appetite. Specifically, prolonged stay at moderate altitude results in a greater dependency on CHO use. Increases in RMR were observed during an acute severe bout of hypoxia, although this was not a consistent effect throughout prolonged exposure and should be further investigated. Altered taste during an altitude stay may influence food preferences, energy intake and subsequent changes in body mass and should be considered an area of future investigation. Higher circulating levels of FFA and TAG, demonstrates a metabolic perturbation from a single, acute severe hypoxic exposure.

TABLE OF CONTENTS

Abbreviations	VIII
List of Tables.....	XII
List of Figures	XIII
List of equations	XVI
Acknowledgements.....	XVII
Declaration.....	XVIII
Conferences preceding's.....	XIX
1.Introduction	1
2. Literature review.....	5
2.1 Hypoxia.....	5
2.2 Classification of differing altitudes.....	7
2.3 Differences in physiological responses to normobaric and hypobaric hypoxia ...	9
2.4 Physiological responses to hypoxia.....	13
2.5 Substrate oxidation at altitude.....	17
2.6 Loss of body mass and alterations in metabolic rate at altitude	28
2.7 The effects of altitude on metabolic rate and basal energy needs	30
2.7.1 Effect of energy balance.....	32
2.8 Hypoxia and fat metabolism	45
2.9 Effect of hypoxia on lipolysis.....	46
2.10 Insulin resistance and sensitivity at altitude	48
2.11 Brown Adipose tissue and Meteorin -like (Metrnl).....	54
2.11.1 Types of adipocytes.....	55
2.12 Brown adipose tissue and thermogenesis	56
2.12.1 "Browning" of White Adipose Tissue	59
2.12.2 Metrnl and fat "browning"	61
2.13 Appetite regulation and body mass ..	65
2.14 Anorexia at altitude/ hypoxia.....	71
2.15 The role of exercise for loss of body mass and changes in cardio-metabolic health	80
2.16 Exercise for loss of body mass using heart rate reserve	85
2.17 Exercise and exposure to environmental hypoxia alters body mass, body composition and metabolic factors	86
2.18 Aim of thesis.....	94

2.18.1 Research questions arising from the literature review	94
2.18.2 Proposed research studies and hypotheses	96
3. General methods.....	98
3.1 Ethics, health and safety	98
3.2 Experimenters	98
3.3 Equipment cleaning and control of substances hazardous to health	98
3.4 Waste disposal	98
3.5 Participants	99
3.5.1 Medical criteria and recruitment	99
3.6 Pre-trial diet and exercise standardisation	99
3.7 Criteria for termination of experiments	100
3.8 Hydration assessment	100
3.9 Urine osmolality	100
3.10 Urine specific gravity	100
3.11 Anthropometric assessment	101
3.11.1 Body mass	101
3.11.2 Body fat percentage	101
3.12 Environmental conditions	101
3.12.1 Ambient laboratory temperature control	101
3.12.2 Controlling the hypoxic environment	101
3.13 Cardiopulmonary measures	102
3.13.1 Douglas bag metabolic gas analysis	102
3.13.2 Online gas analysis	103
3.13.3 Calculations derived from cardiopulmonary measures	103
3.13.4 Peripheral artery oxygen saturation	104
3.13.5 Heart rate	104
3.14 Perceptual scales.....	104
3.14.2 Rating of perceived exertion	105
3.15 Statistical analysis.....	106
3.15.1 Descriptive statistics.....	106
3.15.1.1 Measurement of central tendency – Mean	106
3.15.1.2 Variation of data	107
3.15.1.3 Standard deviation	107
3.15.2 Normality and sphericity of data.....	107
3.15.2.1 Normal distribution	107
3.15.2.2 Sphericity.....	107

3.15.3 Statistical tests of difference.....	108
3.15.3.1 Statistical significance level.....	108
3.15.3.2 Effect sizes.....	108
3.15.3.3 Cohen's D (<i>d</i>).....	108
3.15.3.4 Partial eta squared (η^2).....	109
3.15.3.5 Analysis of Variance (ANOVA).....	109
3.15.3.6 Post-hoc testing.....	110
3.15.4 Reliability statistics.....	110
3.15.4.1 Typical error of measure (TEM).....	110
3.15.4.2 Coefficient of variation.....	110
3.15.4.3 Interclass correlation coefficient.....	110
3.16 Power analysis.....	111
3.17 Phlebotomy and biochemistry.....	111
3.17.1 Cannulation.....	111
3.17.2 Blood plasma separation.....	111
3.17.3 Analysis of FFA.....	111
3.17.4 Analysis of TAG.....	112
3.17.5 Analysis of Meteorin-like (METRNL).....	112
4. Examination of the reproducibility and validity of the MetaMax3X in normobaric hypoxia at rest.....	115
4.1 Abstract.....	115
4.2 Introduction.....	116
4.3 Materials and Methods.....	118
4.4 Results.....	121
4.5 Discussion.....	125
4.6 Conclusion.....	129
5. An acute exposure to $\text{FiO}_2:0.12$ induces alterations in markers of lipid metabolism in a healthy human population.....	130
5.1 Abstract.....	130
5.2 Introduction.....	131
5.3 Materials and Methods.....	132
5.4 Results.....	134
5.5 Discussion.....	137
5.6 Conclusion.....	141

6. The separate and combined effects of acute hypoxia, moderate intensity exercise and lipid ingestion on markers of metabolism.....	142
6.1 Abstract	143
6.2 Introduction	144
6.3 Materials and methods	146
6.4 Results	150
6.5 Discussion.....	157
6.6 Conclusion	162
7. Moderate altitude induces metabolic effects in healthy humans acutely and following an 18 day stay at 3,400m.....	163
7.1 Abstract	163
7.2 Introduction	164
7.3 Materials and methods	166
7.4 Results	170
7.5 Discussion.....	179
7.6 Conclusion	186
8. The effects of a moderate altitude stay on postprandial metabolic parameters	187
8.1 Abstract	187
8.2 Introduction	188
8.3 Materials and methods	191
8.4 Results	193
8.5 Discussion.....	202
8.6 Conclusion	208
9. General Discussion	209
9.1 Overview	209
9.2 Thesis aims	209
9.3 Principle findings	210
9.3 Mechanistic overview	215
9.3.1 Resting metabolic rate	215
9.3.2 Substrate utilisation	217
9.3.4 Blood lipid responses	219
9.3.5 Meteorin-like.....	220
9.3.6 Appetite, taste and subsequent effects of energy intake and energy balance	221
9.4 Practical application of findings	224
9.5 Limitations.....	230
9.6 Directions for future research	231

9.7 Conclusion	233
References.....	235
Appendices.....	254
Informed consent.....	254
Medical Questionnaire.....	255

ABBREVIATIONS

Abbreviation list is limited to terms used across the thesis

AMS	Acute mountain sickness
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BF%	Body fat percentage
BM	Body mass
BMI	Body mass index
BMR	Basal metabolic rate
CHO	Carbohydrate
CHOL	Cholesterol
CI	Confidence interval
cm	Centimetres
CO ₂	Carbon dioxide
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EDTA	Ethylenediaminetetraacetic acid
EE	Energy expenditure
EPO	Erythropoietin
Fe	Expired fraction
FFA	Free fatty acid
FFM	Fat free mass
Fi	Inspired fraction
g	Grams
GI	Glucose
GLUT4	Glucose transporter 4
HACE	High altitude cerebral edema

HAPE	High altitude pulmonary edema
Hb	Haemoglobin
HDL	High density lipoprotein
HH	Hypobaric hypoxia
HIF	Hypoxic inducible factor
HIF α	Hypoxic inducible factor 1-alpha
HOMA	Homeostatic model assessment
HR	Heart rate
HRR	Heart rate reserve
HSL	Hormone sensitive lipase
HVD	Hypoxic ventilatory decline
HVR	Hypoxic ventilatory response
ICC	Intraclass correlation coefficient
IMTG	Intramuscular triglycerides
kg	Kilograms
kcal	Kilocalories
LLQ	Lake Louise questionnaire
LDL	Low density lipoprotein
LM%	Lean mass percentage
LPL	Lipoprotein lipase
m	Metres
MetrnI	Meteorin-like protein
min	Minute
ml	Millilitre
mmHG	Millimetres of mercury
NH	Normobaric hypoxia
np ²	Partial eta squared
O ₂	Oxygen

PaO ₂	Partial pressure of oxygen
PCO ₂	Partial pressure of carbon dioxide
P _{ET} O ₂	End tidal partial pressure of oxygen
P _{ET} CO ₂	End tidal partial pressure of carbon dioxide
PO ₂	Oxygen tension
PRE	Prior to departure
PDK	Pyruvate dehydrogenase kinase
RER	Respiratory exchange ratio
RH	Relative humidity
RMR	Resting metabolic rate
RPE	Rating of perceived exertion
RPM	Revolutions per minute
SpO ₂	Peripheral arterial oxygen saturation
SO ₂	Saturation of oxygen
SBP	Systolic blood pressure
SD	Standard deviation
SNS	Sympathetic nervous system
TAG	Triglyceride
TBW%	Total body water percentage
TCA cycle	Tricarboxylic acid cycle
TEM	Technical error of measurement
TE (CV%)	Technical error as a coefficient of variation
UCP1	Uncoupling protein-1
\dot{V} CO ₂	Carbon dioxide production
\dot{V} _E	Ventilation
\dot{V} O ₂	Oxygen consumption
\dot{V} O ₂ max	Maximal oxygen uptake
\dot{V} O ₂ peak	Peak oxygen uptake

$\dot{V}CO_2$

Carbon dioxide production

WAT

White adipose tissue

LIST OF TABLES

TABLE 2.1: Comparison of atmospheric conditions according to the International Civil Aviation Organization (ICAO) (Ruff and Strughold, 1944) in isobaric conditions (Küpper et al., 2011).

TABLE 2.2: Changes in body weight, body composition, and metabolic parameters in humans following exposure to altitude.

Table 2.3: Similarities and dissimilarities between cold and high altitude (Askew 1995).

TABLE 4.1: Mean \pm SD data for metabolic parameters in conditions of FiO_2 : 0.21 and FiO_2 : 0.12

TABLE 4.2: Mean \pm SD data, and reliability statistics for metabolic parameters using the MetaMax3X and the Douglas bag.

TABLE 5.1: Mean \pm SD data for pre, 30 minute, and post-values for blood markers in the control and hypoxic group exposed to [Fraction of inspired Oxygen FiO_2 : 0.21].

TABLE 5.2: Mean \pm SD data for physiological measures throughout the exposure to hypoxia in the hypoxic group.

Table 8.1: Mean \pm SD data for physiological measures recorded at PRE, POST1 and POST2.

TABLE 9.1: Hypotheses for each study chapter presented in this thesis

LIST OF FIGURES

FIGURE 2.1: The oxygen dissociation curve in a theoretical healthy subject with a “normal blood haemoglobin concentration of 15 g.dL⁻¹” (Collins et al., 2015a).

FIGURE 2.2: Schematic summary indicating the alteration of physiological variables at two altitudes (1,500 – 2,000 m and 3,000 – 3,500 m) (Bärtsch and Saltin 2008).

FIGURE 2.3: Respiratory exchange ratio (RER) during exercise and recovery at a simulated altitude of 2,000 m and at sea-level.

FIGURE 2.4: Known and postulated connections between acute hypoxia, acclimatisation, substrate use and metabolic economy (Braun, 2008).

FIGURE 2.5: Energy metabolism in the skeletal muscle (Horscroft and Murray 2014).

FIGURE 2.6: Representation of the influence of cold and high-altitude environments on energy and fluid balances and resulting physiological consequences (Askew, 1995).

FIGURE 2.7: Fatty acids are released from adipose tissue by lipolysis whilst muscles break down releases amino acids. Fatty acids and some amino acids are converted into ketone bodies by the liver. Ketone bodies and amino acids act as metabolic substrates (Murray and Montgomery 2014).

FIGURE 2.8: Weight gain (A) and food intake (B) over four weeks of cycle hypoxic (8% O₂) and normoxic exposure. Columns represent four days of hypoxia (Quintero et al., 2010).

FIGURE 2.9: An overview of the acute control of brown adipose tissue activity (Cannon, 2004).

FIGURE 2.10: Diagram illustrating the process of white adipose tissue being converted into a “beige” adipocyte with descriptions of the thermogenic process (Ishibashi and Seale 2010)

FIGURE 2.11: The suggested “anti-obesity” effects and “anti-diabetic and anti-dyslipidemic” effects of brown adipose tissue (BAT) (Kim and Plutzky 2016).

FIGURE 2.12: Overview of the major hormonal factors influencing energy balance and the possible interaction between exercise, environmental factors and energy intake (Stensel, 2011).

FIGURE 2.13: Short term regulation of feeding behaviour (Speakman, 2014).

FIGURE 2.14: Relationship between sea-level energy intake and high-altitude energy intake ($\text{kcal}\cdot\text{day}^{-1}$) compiled from studies with ad libitum food intake (Marriott and Carlson, 1996).

Figure 2.15: A summary of the metabolic responses to hypoxia/ high altitude

FIGURE 2.16: Relative increase in energy derived from carbohydrate (CHO) utilisation and decline in energy from oxidation of lipid utilisation as a function of relative aerobic power. At the point of crossover increments in relative exercise intensity results in an increasingly greater dependence on CHO and less dependence on fat (Brooks et al., 1994).

FIGURE 2.17: Change in body fat mass % in normoxia and hypoxia groups following the four week exercise training programme (Wiesner et al., 2010).

FIGURE 2.18: Percentage Change in body fat, body mass index, and body mass between normoxia and hypoxia groups (Haufe et al., 2008).

FIGURE 2.19: Changes in triglycerides (TG), cholesterol (CHOL), high density lipoprotein (HDL) and low density lipoprotein (LDL) between normoxia and hypoxia groups (Haufe et al., 2008).

FIGURE 3.1: Lake Louise questionnaire score (Savoirey et al., 1995).

FIGURE 3.2 Rating of perceived exertion (RPE) scale (Borg et al., 1985).

FIGURE 4.1: Bland-Altman plots of the mean bias (between MetaMax3X testing trials) for metabolic data.

FIGURE 4.2: Bland-Altman plots of the mean bias (between MetaMax3X 1 and Douglas Bag (DB) testing trials) for metabolic data.

FIGURE 6.1: Schematic of experimental design.

FIGURE 6.2: Energy expenditure (EE) and carbohydrate (CHO) and fat (FAT) oxidation in hypoxia and exercise (HYP), normoxia and exercise (NORM) and control (CON) conditions (mean \pm SD).

FIGURE 6.3: Comparison of total energy expenditure between hypoxia and exercise, normoxia and exercise and control conditions.

FIGURE 6.4: Meteorin-like in control, hypoxia and exercise, and normoxia and exercise conditions (mean \pm SD).

FIGURE 6.5: Triglycerides and free fatty acids in control, hypoxia and exercise and normoxia and exercise conditions

FIGURE 7.1: Schematic of study design.

FIGURE 7.2: Body Mass loss and resting metabolic rate in EE/kg/day from PRE to DAY46.

FIGURE 7.3: Body Mass loss and resting metabolic rate in kcal.kg⁻¹day⁻¹ from PRE to DAY46 for males and females separately.

FIGURE 7.4: The contribution of carbohydrate and FAT (%) to resting energy expenditure (B).

Figure 8.1: Schematic of study design.

Figure 8.2: Plasma free fatty acids and triglyceride concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2).

FIGURE 8.3: Glucose concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 AND POST2).

Figure 8.4: Plasma high density lipoproteins, low density lipoproteins HDL, LDL and total cholesterol concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2).

Figure 8.6: RER, CHO oxidation (g.min⁻¹) and Fat oxidation values (g.min⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2).

Figure 9.1: Illustration of the influence of reductions in sensation of taste on subsequent energy intake.

Figure 9.2: Proposed theoretical model of the determining factors of reduced body mass upon exposure to high altitude.

LIST OF EQUATIONS

Equation 3.1: Calculation for metabolic energy expenditure

Equation 3.2: Calculation of respiratory exchange ratio (RER) (Williams et al. 2008).

Equation 3.3: Calculation of oxidation rates of fat and carbohydrate (CHO) (Peronnet and Massicotte 1991)

Equation 3.4: Calculation of the mean

Equation 3.5: Calculation of standard deviation

Equation 3.6: Calculation of skewness

Equation 3.7: Calculation of kurtosis

Equation 3.8: Calculation of Cohen's D (d)

Equation 3.9: Calculation of partial eta squared (η^2)

Equation 3.10: Calculation of typical error of the measure (TEM) (Norton et al. 1996)

Equation 3.11 Calculation of technical error as a coefficient of variation (TE (CV %))

Equation 3.12: Calculation of intra class correlation (ICC)

Equation 4.1: Calculation of limits of agreement 95% confidence intervals.

Equation 6.1: Calculation of age predicted maximum heart rate (HR_{max}) (Tanaka et al., 2001)

Equation 6.2: Calculation of heart rate reserve (HRR) (Karvonen 1957)

Equation 6.3: Calculation of area under the curve. Trapezoid method.

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DECLARATION

I declare that the research contained in this thesis, unless otherwise indicated within the text, is original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed:

A handwritten signature in black ink, appearing to read "B. Duncan". The signature is written in a cursive style with a large initial "B" and a long horizontal stroke at the end.

BENEDICT DUNCAN

DATE: NOVEMBER 2016

CONFERENCES PRECEDING'S

Duncan, B. Richardson, A.J. Watt, P.W. (2015) Metabolic responses to acute hypoxia and moderate intensity normoxic exercise in humans. In. European College of Sport Science (ECSS), Malmö, Sweden, June 2015.

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1. INTRODUCTION

A principal finding from Paul Bert's publication of "La Pression Barometrique" in 1878 was that the observed effects of exposure to low pressure could be attributed to the effects of low partial pressure of oxygen (Bert, 1878). Since then the scientific exploration into the numerous effects of hypoxia on the human body has occurred revealing a body of complex and multi-faceted discoveries. Metabolism can broadly be defined as the collection of all chemical reactions that occur within the cells of the body, largely regulated by hormones secreted by glands in the endocrine system and often separated into the two categories of anabolism and catabolism (Marieb, 1997). This thesis aims to examine the effect of environmental hypoxia on the response of human metabolism to such a stimulus. Specifically the physiological mechanisms linked to weight loss, changes in substrate oxidation and resting metabolic rate (RMR) upon exposure to altitude.

Today, scientific research centred on conditions of hypoxia is fundamental in the attainment of knowledge regarding the aetiology and treatment of medical disorders including the care of the critically ill. Exposure to hypoxia both "real" and simulated is now common practice for the endurance athlete during periods of rest and training in an aim to improve performance, whilst the mountaineer benefits from scientific research regarding the prevention and treatment of acute mountain sickness and its associated conditions. More recently, and central to this thesis, the therapeutic effect of exposure to hypoxia has been observed in an obese and metabolically unhealthy population (Lippl et al., 2010; Schobersberger et al., 2003). These findings have culminated in the suggestion that a novel and effective treatment strategy for obesity may exist through the utilisation of hypoxia alone and in combination with physical activity. Such a treatment is of particular interest and relevance at a time of growing worldwide obesity incidence and a subsequent rise in its associated metabolic conditions.

It is now well documented that in humans at high-altitude (>5,000 m), the maintenance of body mass is extremely difficult if not impossible (Westerterp 2001). With increasing altitude steady reductions in body mass are observed in healthy humans with typical losses of 150-200 g.day⁻¹ (Butterfield 1996). As such, research has continued in an attempt to better understand the underpinning physiological mechanisms for changes in body mass and composition upon exposure to environmental hypoxia/altitude. A substantial section of the research in this area is focused upon the magnitude and underpinning mechanisms of lean mass loss and strategies to prevent this loss of lean tissue or fat free mass (FFM) in order to maintain performance and health in the mountaineer or sojourner at altitude. More recently, a growing amount of literature has been published with regards to the use of reduced oxygen availability both with and independent of exercise, in an attempt to harness the cachexic effects of environmental hypoxia/altitude (Netzer et al. 2008; Wiesner et al. 2009). Such effects include, amongst other findings, reduced body fat, improved insulin sensitivity, lowered blood pressure and improved exercise tolerance. The growth in this research topic coincides with a

continued increase in the prevalence of obesity and related cardio-metabolic disorders (Haslam. 2005 in WHO report 2010).

Obesity is defined as a condition of excess body fat which is associated with adverse health consequences and negative effects on mortality and is caused by a complex interaction between environmental and genetic factors (WHO. 2010). Currently, more than 25% of the adult population and 14% of children (aged 2-10 years) are reported to be obese in the UK (Zaninotto et al., 2006). Globally the World Health Organization estimates that over 1.9 billion people worldwide are overweight and more than 600 million adults are obese with this number likely to rise to 700 million within 5 years. Adult obesity increases the risk of hypertension, stroke, respiratory problems, gall bladder disease, osteoarthritis, sleep apnoea and certain cancers (Druce and Bloom 2006). Individuals with a body mass index (BMI) within the “overweight” category have a 20% - 40% higher mortality rate compared to healthy weight adults and those individuals who fall in to the “obese” category have a 200% – 300% higher mortality rate compared to healthy weight adults (Laskowski, 2012). Mortality rate is further increased in individuals with a BMI > 40 (Finkelstein et al., 2012). As such effective prevention and treatment strategies to combat the continued rise and associate complications of obesity are of great importance. Integral to this thesis is the physiological association between the described effects of hypoxia and its potential use as an anti-obesity therapy. Although much of the reviewed research has been conducted on “normal” weight humans and rodents, potential links are highlighted within this thesis where appropriate.

Although largely a function of negative energy balance, in part due to inadequate energy intake, a multitude of influencing factors for loss of body mass are present at altitude. Proposed explanations include anorexia, loss of body water, altered satiety hormones and an elevation in energy needs, primarily from basal metabolic rate (Butterfield et al. 1996). Acute and longer term studies have both been conducted in the area and the literature is comprised of a mixture of studies using both terrestrial and simulated altitude. Also linked with alterations in body composition is the effect of reduced oxygen availability on the body’s preference for substrate oxidation (Hall et al. 2012; Brooks et al. 1991).

Further information is required in order to better understand the effect of acute hypoxia on alterations in metabolic rate and how these alterations, when experienced repeatedly over a prolonged intervention, contribute to a meaningful difference in body mass. A second important area of future research is the effects of reduced oxygen availability combined with physical activity on the “browning” of adipose tissue and the subsequent potential in increased total energy expenditure. Furthermore, a greater understanding of the effect of high altitude exposure on contributors to appetite regulation and subsequent energy intake would aid in strategies to both prevent unwanted loss of body mass in athletes, mountaineers and holiday makers as well as for individuals utilising hypoxia as a tool for weight loss and improved metabolic health.

This introduction has outlined the commonly reported loss of body mass upon exposure to high altitude in conjunction with a multitude of contributing metabolic and environmental factors. There is a clear need for further research in regards to the use of hypoxia in the acute, intermediate and chronic term with relation to changes in metabolic factors contributing to changes in body mass. This thesis is therefore presented in the following chapters;

CHAPTER 2 reviews the literature which will highlight the physiological response of humans to hypoxia both real and simulated. The review will subsequently summarise the most relevant published literature with regards to the effect of reduced oxygen availability of differing durations on body mass, body composition, metabolic rate, substrate utilisation, the metabolism of fat and markers of appetite. The use of hypoxia coupled with exercise will also be examined as will the existing work on the effects of differing environmental conditions on brown adipose tissue. Finally, an overall summary of the most pertinent studies will be presented and topics of future research with regards to the physiological effects of hypoxia on human metabolism will be suggested.

CHAPTER 3 describes the common methods used across multiple experimental chapters.

CHAPTER 4 presents the first experimental chapter whereby the validity and reproducibility of an online gas analyser (MetaMax 3X) during acute exposure to environmental hypoxia ($FiO_2:0.12$) is described. This chapter informs future chapters of the similarity in measured ventilatory parameters during acute rest to severe normobaric hypoxia between two commonly practised methods.

CHAPTER 5 describes the findings from the second study in which the metabolic responses to an acute resting normobaric hypoxic exposure ($FiO_2:0.12$) were investigated. The effects of reduced oxygen availability on measures of resting metabolic rate, substrate use, and circulating plasma levels of free fatty acids (FFA) and triglycerides (TAG) are described in a group of apparently healthy individuals.

CHAPTER 6 is an experimental chapter that incorporates the acute resting exposure to hypoxia described in study 2 (Chapter 5). This was done in order to elucidate the independent and combined effects of reduced oxygen availability and a bout of exercise in normoxia on metabolic responses following a high fat meal.

CHAPTER 7 presents the fourth experimental study in which the acute and lasting metabolic responses to an intermediate term stay at moderate hypobaric hypoxia (3,400m) are described. This chapter determines the effect of an 18-day stay in Cusco, Peru on measures of body mass, body composition, resting metabolic rate, and substrate use at three time points throughout the altitude stay and upon one and four weeks return to sea-level. The study also presents the effect of moderate altitude living on sensations of taste, feelings of appetite during the sojourn and the lasting effects on Meteorin-like (Metrnl) upon return to sea-level as an indicator of “browning” of adipose tissue and its potential to contribute to increased whole body EE.

CHAPTER 8 describes the findings from the final experimental study in which the metabolic responses to a high fat meal following an intermediate length stay (18-day) at moderate altitude (3,400 m) are investigated. This study incorporates the same meal ingested in study 3 (Chapter 6) at three time points; one prior to the altitude stay and two upon return to sea-level at one and four weeks post-return during which the response of FFA, TAG, Insulin, substrate use and resting metabolic rate were evaluated.

CHAPTER 9 discusses the individual and cumulative findings of all experimental chapters to form the general discussion. The general discussion contextualises the findings towards the use of exposure to environmental hypoxia and exercise as a potential therapeutic tool for individuals with metabolic disease. This chapter closes by discussing potential future directions for research in the field, in addition to reviewing the practical application of the data presented.

2. LITERATURE REVIEW

2.1 HYPOXIA

Oxygen enters the body via the lungs, is transported via the blood to the tissues and is consumed to provide energy for metabolism. This delivery of oxygen by arterial blood to the tissues has a number of determinants including the concentration, saturation and partial pressure of blood oxygen along with the haemoglobin concentration and cardiac output (Collins et al. 2015b). The oxygen concentration of arterial blood is dependent on several factors including the partial pressure of inspired oxygen, the adequacy of ventilation and gas exchange, the concentration of haemoglobin and the affinity of haemoglobin molecule for oxygen (Collins et al. 2015b). Delivery of oxygen to the tissues is the product of arterial oxygen content and cardiac output and as such can be compromised as much by a low haemoglobin concentration or cardiac output as by a fall in the saturation of blood oxygen. Put simply; Oxygen delivery is dependent on oxygen availability, the ability of arterial blood to transport oxygen and tissue perfusion (McLellan and Walsh 2004). A defect or disruption at any point in this system can result in abnormal oxygenation and may lead, in extreme cases, to tissue damage or death of an organism. Less extreme disruptions in this system lead to a number of alterations in human physiology both during and following reduced levels of oxygen (Pierson, 2000) commonly reported during cardiovascular diseases and upon exposure to altitude. The reduced oxygen availability at high altitude presents significant challenges to humans residing there or to those who are exposed acutely. As such, hypoxia defined as a state of oxygen deficiency within the body's tissues, is often experienced despite an adequate perfusion of tissues by blood and has systematic effects throughout the body (Dempsey and Morgan 2015; Pierson 2000).

A number of definitions are used in relation to hypoxia and its effect on the human body. Hypoxemia is defined as inadequate, subnormal or deficient oxygenation of arterial blood, also described as the decreased oxygen tension (PO_2) in the blood below normal range (Pierson, 2000). Whereas the term anoxia is defined as an abnormally low or an absence of oxygen in the tissues of the body that may arise from lack of oxygen from inspired gases or arterial blood (Pierson, 2000). The latter term generally refers to a more severe state of oxygen deficiency often with an implication of irreversible damage. Although several subtypes of hypoxia have been described including; "anaemic hypoxia", "hypoxic hypoxia", "Ischemic hypoxia", "Oxygen affinity hypoxia" and "stagnant hypoxia" (Pierson, 2000) the term hypoxia will be used throughout this review of literature to describe environmental conditions in which a reduced oxygen supply is available compared to that at sea-level.

Environmental hypoxia, either in a hypobaric or normobaric chamber or at high altitude decreases the partial pressure of arterial oxygen (PaO_2) (Bert 1878;West and Richalet 2013). Normobaric hypoxia is brought about from a reduced fraction of inspired oxygen (FiO_2) and an unchanged barometric pressure. Hypobaric hypoxia, alternatively, is induced through a reduced barometric pressure with no change in FiO_2 (Bert 1878;West and Richalet 2013). The "hypoxic dose" in these simulated conditions

is calculated by a combination of barometric pressure and inspired fraction of oxygen (Conkin and Wessel 2008). Exposure to hypobaric hypoxia can be achieved through specially designed hypobaric chambers and through ascent to altitude. A potentially advantageous aspect of the use of simulated altitude for laboratory experimentation is that extraneous variables can be more easily controlled in comparison to “true” altitude.

The deficiency of oxygen in arterial blood is commonly defined in relation to the oxy-haemoglobin dissociation curve. The oxy-haemoglobin dissociation curve is a graphical representation of the relationship between the saturation of oxygen and the partial pressure of oxygen and aids in the understanding of the principles of oxygen delivery (Figure 2.1). The saturation of oxygen (SO_2) represents the overall percentage of haemoglobin binding sites which are occupied by oxygen and gives information regarding the amount of oxygen that is available to the metabolising tissues (Collins et al. 2015a) provided that the circulatory and haemoglobin function is normal. The partial pressure of oxygen (PaO_2) represents the pressure which would be exerted by oxygen if it alone occupied the volume that the combination of gases it is a constituent of occupies (Collins et al. 2015a). Although not linear, an increase in PaO_2 occurs in line with an increase in SaO_2 .

Upon initial exposure to altitude a rightward shift in the dissociation curve occurs representing lower oxygen saturation for a given PO_2 and highlighting the effect of reduced oxygen availability (Naeraa et al., 1966). A rightward shift in the dissociation curve also occurs in response to an increase in the partial pressure of carbon dioxide (PCO_2), greater acidity and higher temperature in the metabolising tissues (Naeraa et al., 1966). A shift to the right implies a reduction in the affinity of the blood for oxygen, an advantageous response in such situations, as it facilitates the unloading of oxygen from haemoglobin in the tissues (Collins et al., 2015a). During the passage of blood through the pulmonary capillaries the reverse occurs aiding the uptake of oxygen due to a greater affinity for oxygen. Physiological alterations that occur in response to ascent to altitude or exposure to an environment of hypoxia do so predominantly due to changes in the partial pressure of oxygen, changes in the oxygen saturation or content of blood and the interplay between these two factors.

Studies in which pulse oximetry has been compared with measured arterial haemoglobin saturation (Lebecque et al., 1991) have reported values that closely correlate with standard errors of the estimate of 3.2 – 4.1%. It is however suggested that error increases with decreasing saturations with progressively larger errors below 80% saturation. In the current thesis maximum desaturation values of $81 \pm 4\%$ were observed.

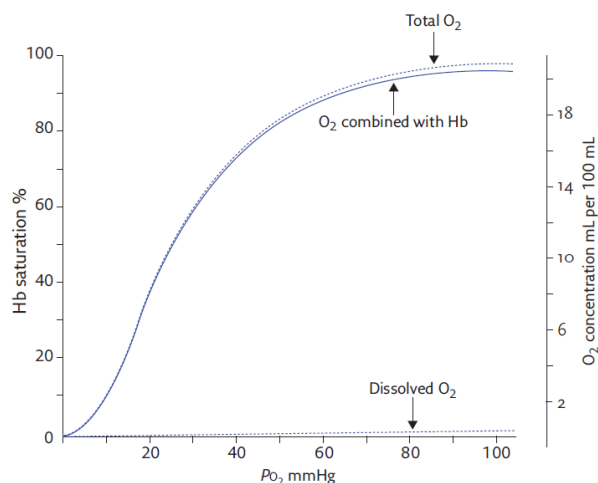


FIGURE 2.1: The oxygen dissociation curve in a theoretical healthy subject with a “normal blood haemoglobin concentration of 15 g.dL⁻¹” (Collins et al., 2015a).

2.2 CLASSIFICATION OF DIFFERING ALTITUDES

Work from Bartsch and Saltin (2008) define altitudes based on the effects of acclimatisation, performance and well-being in healthy individuals; near sea-level has been proposed as altitudes of between 0 - 500 m at which no altitude –related effects are observed. Low altitude is considered to be from 500 – 2,000 m above sea-level at which relevant performance impairments are possible particularly above 1,500 m although these effects can usually be completely overcome by acclimatisation. No altitude related effects on well-being are generally seen at low altitude. Moderate altitude (between 2,000 – 3,000 m) may induce effects on well-being in non-acclimatised individuals as well as sleep disturbance and the occurrence of acute mountain sickness (AMS). Maximum aerobic performance is significantly decreased at this level but can usually be restored largely through acclimatisation. Significant erythropoietic response occurs within three to four weeks at moderate altitude. High altitude (between 3,000 – 5,500 m) induces the occurrence of AMS in a large number of non-acclimatised individuals during the first days of exposure with susceptible individuals at the risk of developing high altitude pulmonary edema (HAPE) above 3,000 m and high altitude cerebral edema (HACE) above 4,000 m. High altitude will significantly reduce athletic performance regardless of acclimatisation. Extreme altitude (> 5,500 m) appears to be the greatest elevation for long term adaptation in humans.

Within published scientific literature regarding the effects of reduced oxygen availability on human physiology differing approaches are used to categorise the severity of hypoxia. These classifications include; i) “altitude above sea-level” which is usually described in metres (m) or feet, ii) %O₂, which refers to the concentration of oxygen in ambient air at sea-level pressure as a percentage, iii) partial pressure of oxygen (pO₂) which has previously been described in this review and is presented in millimetres of mercury (mmHg) and iv) atmospheric pressure which is also described in mmHg (Küpper

et al., 2011). Research carried out in the field at “true” altitude often describe the environment with regards to the elevation above sea-level whereas studies in which simulated altitude is used usually present the %O₂ or the fraction of inspired oxygen (FiO₂) as a means of classification. Physiological differences exist in humans following exposure to true and simulated altitude which will be discussed in this review of literature. The corresponding values between altitude above sea level, %O₂, atmospheric pressure and pO₂ are presented in Figure 2.2. These values should be used with caution however as both %O₂ and altitude have been described as less exact than pO₂ (Küpper et al., 2011). Nevertheless resulting errors are considered small and knowledge regarding the comparative values is important and useful for the interpretation of the hypoxia induced physiological alterations across studies presented in the previous literature.

TABLE 2.1: Comparison of atmospheric conditions according to the International Civil Aviation Organization (ICAO) (Ruff and Strughold, 1944) in isobaric conditions (Küpper et al., 2011).

(Corresponding) Altitude (m)	Atmospheric pressure (mm Hg)	pO ₂ (mm Hg)	%O ₂ , isobaric conditions, and sea level
0	760.0	158.8	20.9
500	716.0	149.6	19.7
1000	673.8	140.8	18.5
1500	634.0	132.5	17.4
2000	596.0	124.6	16.4
2500	560.0	117.0	15.4
3000	525.8	109.9	14.5
3500	493.0	103.0	13.6
4000	462.0	96.6	12.7
4500	432.6	90.4	11.9
5000	404.8	84.6	11.1
5500	378.6	79.1	10.4
6000	353.6	73.9	9.7
6500	330.0	69.0	9.1
7000	307.8	64.3	8.5
10 500	183.0	38.2	5.0
12 900	123.5	25.8	3.4

Notes: Corresponding altitude in metres (m), atmospheric pressure in millimetres of mercury (mmHg), partial pressure of oxygen (pO₂) and percentage of oxygen (%O₂)

2.3 DIFFERENCES IN PHYSIOLOGICAL RESPONSES TO NORMOBARIC AND HYPOBARIC HYPOXIA

OVERVIEW

Debate exists regarding the physiologically evoked differences of exposure to normobaric compared to hypobaric hypoxia. This relates to a simulated normobaric hypoxia and both a simulated hypobaric environment and terrestrial altitude. Much research regarding the effects of reduced oxygen availability has been conducted in the “field” at high altitude in hypobaric hypoxia. Such work is present on various topics including, amongst others, the optimisation of training for athletes (Bailey and Davies 1997; Girard et al. 2013; Millet et al. 2013), the prediction and treatment of acute mountain sickness (Grant et al., 2002), the pathogenesis of medical conditions characterised by hypoxia (Grocott et al., 2007), the treatment of metabolic and cardiovascular conditions (Palmer and Clegg 2014) and the effect of residing at high altitude on mortality (Burtscher, 2014). Such studies however, present numerous challenges to the researcher, particularly the logistical aspects of collecting data in the field at altitude. As such, much research regarding the effects of hypoxia is also conducted in a simulated environment in an attempt to replicate high-altitude conditions within a setting more conducive to data collection. The recent development and increase in availability of “altitude chambers” has also resulted in increased use of hypoxia for both scientific study and for recreational purposes including fitness training. The replication of high altitude conditions is done so in either a hypobaric hypoxic environment or a normobaric hypoxic environment. The interchangeable nature between hypobaric and normobaric hypoxia and their effect on physiological responses in humans however is not proven (Girard et al. 2012; Millet et al. 2012; Coppel et al. 2015; Richard et al. 2014). The following section will attempt to highlight the major suggested variations between the two environments with regards to physiologically evoked differences and provide information regarding the practical implications of such differences.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

Hypoxia, through the lowering of the barometric pressure or by the reduction of the percentage of inhaled oxygen, elicits physiological responses in humans. Independent effects of hypobaria apart from hypoxia have however been previously observed, particularly when exposure to such conditions is sudden, an effect initially demonstrated in divers and aircrew but may now be relevant to chamber studies (Di Giulio and West 2013).

In a recent systematic review investigating the differences in crossover studies between hypobaric and normobaric hypoxia it was suggested that confounding factors including time spent in hypoxia, the temperature, humidity and the relatively small sample sizes limits the available conclusions from such comparisons (Coppel et al., 2015). Nevertheless reported differences in physiological parameters of minute ventilation (Faiss et al., 2013), AMS related factors (Roach et al., 1996), peripheral oxygen saturation (SpO_2) (Savoirey et al., 2007, 2003), cardiovascular variables (Savoirey et al., 2007, 2003;

Tucker et al., 1983) and Nitric oxide levels (Hemmingsson and Linnarsson 2009; Faiss et al. 2013) seem to support the notion of true differences between normobaric (NH) and hypobaric (HH) hypoxia.

Regarding ventilatory parameters, altered values of minute ventilation have been observed in HH in a number of studies when direct comparisons have been made to NH (Faiss et al., 2013; Loeppky et al., 1997; Savourey et al., 2003; Tucker et al., 1983) with differences of up to 4 L.min⁻¹ reported (Loeppky et al., 1997). Specifically Loeppky et al., (1997) observed that at an equivalent environment of FiO₂: 0.142 during a 10 hour exposure at rest initial values of \dot{V}_E were similar between HH and NH which equated to a 38% increase compared to ambient measures at normobaric normoxia. However following prolonged exposure differences equating to an average of 26% in values of \dot{V}_E were reported at three, six and nine hours with lower values observed in HH compared to NH. The greatest difference between conditions was observed at three hours with an average discrepancy of 39% that were mainly due to a decline in \dot{V}_E in the HH environment. A suggested mechanistic explanation for lower values at HH is the lower elimination of carbon dioxide (CO₂) in relative terms at altitude compared to normobaric conditions due to a reduction in inspired gas density which may in turn reduce the work of breathing (Loeppky et al., 1997).

Other comparative studies have however observed no differences in the same measure (Miyagawa et al., 2011; Savourey et al., 2007). In accordance with lower values of minute ventilation in HH measures of tidal volume have also been seen to be lower in a number of studies (Faiss et al., 2013; Savourey et al., 2007, 2003; Tucker et al., 1983) with differences of up to 0.9 L reported (Faiss et al., 2013) as have measures of alveolar ventilation (Loeppky et al., 1997). A discrepancy in values of breathing frequency has also been seen in comparative studies however both increases (Savourey et al., 2007, 2003) and decreases (Tucker et al., 1983) are described in HH compared to NH.

Some evidence exists that values of SpO₂ measured by pulse oximetry are lower (- 4.08%) in HH compared to NH during exposures of short duration (Savourey et al., 2007, 2003) suggesting a more pronounced effect on such parameters from a hypobaric stimulus. Furthermore measured values of acute mountain sickness have been seen to be more severe in HH than NH (Roach et al., 1996). Interestingly however these same observations with regard to SpO₂ do not appear to be consistent in studies of longer duration (Faiss et al., 2013; Loeppky et al., 1997; Roach et al., 1996; Tucker et al., 1983). Such findings highlight the complex nature of the evoked differences between environments and seem to suggest that observed differences between conditions are, in part, dependent upon the measured outcome and the length and severity of exposure. Length of exposure is deemed important due to the different response rates for adaptation to hypoxia of different physiological systems. Studies of short duration therefore may not be sufficient for differences between normobaric and hypobaric hypoxia to be elicited or alternatively may elicit transient initial differences that cease following prolonged exposure. This is demonstrated by work from both Loeppky et al. (1997) who reported no differences in ventilation rates after 30 or 60 minutes when comparing conditions but did

observe a difference after three hours of exposure, and work from Savourey et al., (2003) who highlighted initial differences for measures of end tidal partial pressure of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) which subsequently showed no difference following prolonged exposure.

Cardiovascular related parameters are inconsistent in the comparative response between HH and NH. Previous research has reported both higher (Savourey et al. 2003; Self et al. 2011; Tucker et al. 1983) and lower (Basualto-Alarcón et al., 2012) measures of heart rate (HR) in HH as well as no differences between environmental conditions (Faiss et al., 2013; Miyagawa et al., 2011; Savourey et al., 2007). Measures of blood pressure (BP) have been reported as lower in HH (Tucker et al., 1983) although the majority of comparative studies observed no differences (Faiss et al., 2013; Miyagawa et al., 2011) whereas “sympathetic drive” measured through electrocardiogram (ECG) was found to be higher in HH compared to NH (Basualto-Alarcón et al., 2012). Such findings make it difficult to draw definitive conclusions with regards to CV parameters based on the above studies although an initial increase in HR at HH compared to NH is suggested. Other physiological variables including plasma pH values and nitric oxide are also inconsistent. Measures of plasma pH have been recorded as higher in HH (Savourey et al., 2007, 2003; Tucker et al., 1983) but the reverse was also found in a separate investigation (Faiss et al., 2013). Increases in pH values of 0.032 at HH were the largest observed between conditions (Tucker et al., 1983). Exhaled levels of nitric oxide (NO) have been seen to be lower in HH than in NH (Hemmingsson and Linnarsson 2009; Faiss et al. 2013) with observed differences of up to 6.8 mmHg reported.

Other studies have demonstrated practical implications of differences between the hypobaric and normobaric environments. Particularly there is a growing body of research regarding the variances in endurance performance following training and exposure to a normobaric compared to a hypobaric environment (Bailey and Davies 1997; Fulco et al. 2013). Furthermore differences in endurance performance trials undertaken in the two settings are also observed (Beidleman et al. 2014; Saugy et al. 2015). Such studies demonstrate cycling time trial performance to be impaired in hypobaric compared to an equivalent normobaric hypoxia. Specifically work from Saugy et al. (2015) investigated the differences in time trial performance in trained male participants and reported an increase in time to complete the trial in both a normobaric ($+24.1 \pm 9.6\%$) and hypobaric ($+33.2 \pm 12.4\%$) environment when compared to normobaric normoxia. Additionally time trial performance was hindered in hypobaric compared to normobaric conditions at an equivalent altitude of 3,450 m by 7.5%. This reduction in performance was seen in conjunction with a reduction in SpO_2 ($79.2 \pm 3.4\%$ in NH vs. $75.9 \pm 5.9\%$ in HH) (Saugy et al., 2015).

A suggested mechanism for performance impairment at altitude compared to normoxia is a reduction in maximal oxygen uptake ($\dot{V}O_2 \text{ max}$) with average decrements of 7.7% per 1000 m increase in altitude observed (Chapman and Emery 1999; Mollard et al. 2007; Wehrlin and Hallén 2006). A reduction in $\dot{V}O_2 \text{ max}$ has been closely linked with a decrease in arterial oxyhaemoglobin saturation which supports

the findings from Saugy et al. (2015). Work from Beidleman et al., (2014) also reported decreased cycling time trial performance upon exposure to hypobaric hypoxia in comparison to normobaric hypoxia equivalent to 4,300 m. These observations were reported despite similar cardiorespiratory responses and thus no observed differences in ventilatory or cardiovascular measures. Similar responses in haemoglobin mass have recently been observed following an 18 day live high-train low training camp in both stimuli (+4.1 – 4.5%) (Hauser et al., 2015). Suggested explanations for the differences in endurance performance are thus difficult to explain in the study from Beidleman however it has been suggested that due to “borderline” differences in measures of SpO₂ a larger sample size may have been needed to observe physiological variances.

With regards to acute mountain sickness there is also some evidence to suggest that greater severity of AMS symptoms occur in HH in comparison to NH (Loeppky et al., 2005) although other studies have also reported no differences (Richard et al., 2014b). In a study from Roach et al (1996) nine participants displayed significantly higher AMS scores in HH compared to NH following nine hours exposure to an equivalent altitude of 4,564 m (Roach et al., 1996). Interestingly acclimatisation prior to travel to altitude reduced symptoms of AMS when undertaken in a hypobaric environment but not in a normobaric environment (Fulco et al., 2013) suggesting practical implications exist with regards to using normobaric hypoxia as a preparation tool prior to high altitude travel. In an earlier study from Fulco et al. (2011) the effect of repeated exposures to normobaric hypoxia (FiO₂: 16.2 – 14.4) during sleep were investigated on the ventilatory acclimatization response and scores of AMS upon exposure to terrestrial altitude (five days at 4,300 m). It was observed that upon awakening hours only during the subsequent stay at altitude did those participants exposed to the intermittent normobaric exposures demonstrate higher SpO₂ ($80 \pm 1\%$ vs. $76 \pm 1\%$) and lower AMS values ($0.34 \pm 0.12\%$ vs. $0.83 \pm 0.14\%$) compared to individuals who did not undertake pre-travel normobaric exposures. Interestingly there were no differences in AMS values throughout the rest of the day between groups suggesting that any acclimatisation achieved via the normobaric exposures was expressed primarily during sleeping hours at altitude. These findings are in slight contrast to earlier investigations (Beidleman et al., 2003, 2008; Katayama et al., 2001) which have demonstrated acclimatisation results at altitude following pre-travel hypobaric exposures characterised by exercise arterial oxygen saturation and the hypoxic ventilatory response. Such findings again suggest a greater acclamatory response from hypobaric exposures compared to normobaric upon exposure to “true” altitude.

SUMMARY

Following work from Savourey et al. (2003), during which a 40 minute exposure to both normo and hypobaric hypoxia was investigated, the author concluded that at an equivalent PO₂ equal to 120 hPa; hypobaric hypoxia induces greater hypoxemia, hypocapnia, blood alkalosis and a lower O₂ arterial saturation. These differences were suggested to be specific responses to the hypobaric nature of the hypoxia and as such mechanistic explanations for observed differences between normobaric and

hypobaric hypoxia have been suggested with the difference in pressure highlighted as the principle confounder between the two conditions (Coppel et al., 2015). More specific suggestions as to the evoked physiological differences include increased alveolar dead-space (Loeppky et al., 1997), altered fluid permeability (Roach et al., 1996), changes in chemo-sensitivity (Savoirey et al., 2003) and differences between ventilation and perfusion (Loeppky et al., 1997). Moreover the reduction in gas density with hypobaria is a likely contributor to observed differences leading to reduced work of breathing and alterations in gas diffusion rates (Di Giulio and West 2013). Further confounding factors and possible explanations for observed discrepancies between normobaric and hypobaric conditions include; ambient temperature, humidity, study design and sample size.

Taken together the described investigations demonstrate physiological differences between different “types” of hypoxia suggesting that the two are not entirely interchangeable. It is proposed that a “greater” physiological response is induced via a hypobaric environment (Saugy et al., 2015). Observations within the literature are however inconsistent and further work is required to determine the extent of the interchangeable nature between normobaric and hypobaric hypoxia and to more clearly establish the underpinning physiological differences induced by the two conditions.

2.4 PHYSIOLOGICAL RESPONSES TO HYPOXIA

OVERVIEW

Alterations in oxygen partial pressures induce a host of physiological responses in humans which occur in an attempt to adapt to the conditions of hypoxia. The human response to hypoxia is characterised through immediate adjustments and acclimatisation by systemic changes in cardiovascular, respiratory and hematopoietic physiology which have subsequent effects on oxygen transport (Petousi and Robbins 2014). The threshold altitude and magnitude of the responses to hypoxia vary between the different physiological systems and also show a large inter-individual response (Bärtsch and Saltin 2008). Ensuring the oxygen supply to tissues and organs of the body with an optimal oxygen tension of the arterial blood is the key aim in acclimatisation to altitude (Bärtsch and Saltin 2008). Differing physiological responses occur in response to differing lengths of exposure to hypoxia. The current section will therefore highlight the developing physiological responses of humans from short to longer term exposure to environmental hypoxia.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

Acute responses to elevated altitude are characterised by an activation of the sympathetic nervous system bringing with it an increase in heart rate, a decrease in stroke volume and induced vascular system alterations. Hypoxia constricts rather than dilates human pulmonary vasculature (Motley and Cournaud 1947 in Petousi and Robbins 2014). Hypoxic pulmonary vasoconstriction leads to a subsequent increase in blood pressure, as a response to the constriction of arterioles, and increase in resistance to blood flow following the release of the hormone adrenaline from the adrenal glands

(Motley and Cournand 1947 in Petousi and Robbins 2014). Pulmonary artery pressure starts to rise at an altitude of approximately 3,000 m in healthy individuals. An increase in pulmonary artery pressure of about 60% (Bartsch et al. 1991) and an increase in systemic blood pressure were observed following a 20-day stay at an altitude of 4,300 m due to continued increased sympathetic activity (Reeves et al., 1992). Significant changes in blood pressure at moderate altitude however are considered unlikely (Bärtsch and Saltin 2008). Chemoreflex activation of the sympathetic nervous system occurs upon acute exposure to altitude related to influences from oxygen sensitive chemoreceptors in the carotid body and brainstem. A prolonged sympathetic “overactivity” at hypoxia following four weeks at 5,260m has been directly measured compared to sympathetic activity at sea-level (Hansen and Sander 2003). These findings were observed in conjunction with increased mean arterial blood pressure. Increased blood pressure upon exposure, particularly in individuals with an already raised blood pressure, is therefore a considerable risk that must be investigated further prior to long term programmes incorporating exercise and hypoxia being advised. This is of particular importance for the thesis within which the potential beneficial effects of hypoxia are used as a rationale to suggest positive therapeutic effects may be gained in an unhealthy population.

Maximal heart rate is reduced upon ascent to altitude and decreases with increasing altitude despite a continuing increase in sympathetic activity (Hansen and Sander 2003). Mechanistic factors for this response include an enhanced parasympathetic neural activity (Boushel et al., 2001) and a possible down regulation of the beta-receptors (Richalet et al., 1988).

Debate exists however regarding the altitude and time frame at which a reduction in maximal heart rate occurs with previous findings reporting, following an acute exposure to 4,000 m, no reduction in either cardiac output or maximal heart rate highlighting maintenance of stroke volume (Stenberg et al., 1966). It therefore appears that exposure for a number of hours are required before a reduction in maximal heart rate is elicited with a continued decrease seen in the days post-initial exposure (Lundby et al., 2001; Saltin, 1996). It is also clear that there is considerable inter-individual variation in the response of heart rate to moderate altitude (Friedmann et al., 2005). At more severe altitudes the reduction in maximal heart rate has been suggested to be caused by a reduction in the work of the heart and the demand for a high myocardial oxygen consumption (Bärtsch and Saltin 2008) although this explanation is debated. An explanation as to the reason for the lowering of the maximal heart rate has been suggested as a lowering of heart rate will mean a lower pulmonary flow and an increased mean transit time for the red blood cells passing through the lungs, particularly at peak exercise. This will therefore allow for optimal oxygenation of the blood whilst passing the lungs and thereby secures an optimal saturation of arterial blood (Boushel et al., 2001).

A rise in red cell mass accompanied by elevated haemoglobin (Hb) concentration and haematocrit is a hallmark of high altitude hypoxic exposure which is associated with a rise in blood erythropoietin (EPO) levels (Richalet et al., 1994). Initially however increases in haemoglobin concentration occur due

to a rapid reduction in plasma volume and a delayed effect of erythropoiesis (Lundby et al., 2007). Arterial hypoxemia, or low blood oxygen, and short term sustained EPO production can result from altering inspired gas composition and increased altitude once a threshold of 2,100 m -2500 m is reached (Ge et al., 2002). This hematologic acclimatisation response facilitates the restoration of normal blood O₂ content and improves tissue oxygenation despite lowered arterial PO₂. Increases in EPO concentrations in blood occur between 90 – 120 minutes following exposure to reduced PO₂ and continue to increase during the first 24 - 48 hours before declining over the following days to weeks (Eckardt et al., 1989; Ge et al., 2002; Richalet et al., 1994). Significant increases in red blood cell mass may occur after three weeks at a minimum altitude of 2,100 m with greater increases at greater altitudes (Schmidt and Prommer 2008). The combination of increased oxygen carrying capacity and ventilatory acclimatisation may lead to a greater oxygen content of arterial blood compared to pre-acclimatisation at sea-level (Calbet et al., 2004).

An immediate rise in ventilation is the most obvious response of human lowlanders exposed to acute hypoxia. This is followed by more complex time-dependent changes if exposure is prolonged (Powell et al., 2001). This altitude induced response in ventilation, commonly referred to in the literature as the hypoxic ventilatory response (HVR) (Duffin, 2007), is outlined as one of the most important components of the high altitude acclimatisation process and characterised by a progressive increase in ventilation and PaO₂ and a subsequent fall in PaCO₂. The initial increase in ventilation, driven by the peripheral chemoreceptors, located in the arch of the aorta and carotid body, is followed by the continuation of increased ventilation over 10 - 14 days due to the increasing sensitivity of the peripheral chemoreceptors. This results in an increased arterial oxygen saturation during the first 14 days at a given altitude (Bärtsch and Saltin 2008). A decline in ventilation, termed the hypoxic ventilatory decline (HVD), occurs within minutes after the initial rise prior to acclimatisation (Ainslie et al., 2013). Although exact reasons for the ventilatory decline are uncertain suggestions include a decreased chemoreceptor sensitivity, elevations in coronary blood flow and or elevations in neural stimulus (Ainslie et al., 2013). The time course of ventilatory acclimatisation is dependent on the altitude and hence the PO₂ and the intrinsic hypercapnic ventilatory response. It has been suggested that full acclimatisation can take up to four to six weeks at an altitude of 5,050 m, two to three weeks at 4,000 m and up to four days at 3,000 m. Importantly acclimatisation does not result in PaO₂ being restored to sea-level values nevertheless a progressive increase in ventilation and PaO₂ and a subsequent fall in PaCO₂ are important features in altitude acclimatisation. Figure 2.3 summarises the acute and longer term response of a number of physiological markers in humans exposed at rest to two differing altitudes (1,500 m and 3,000 m).

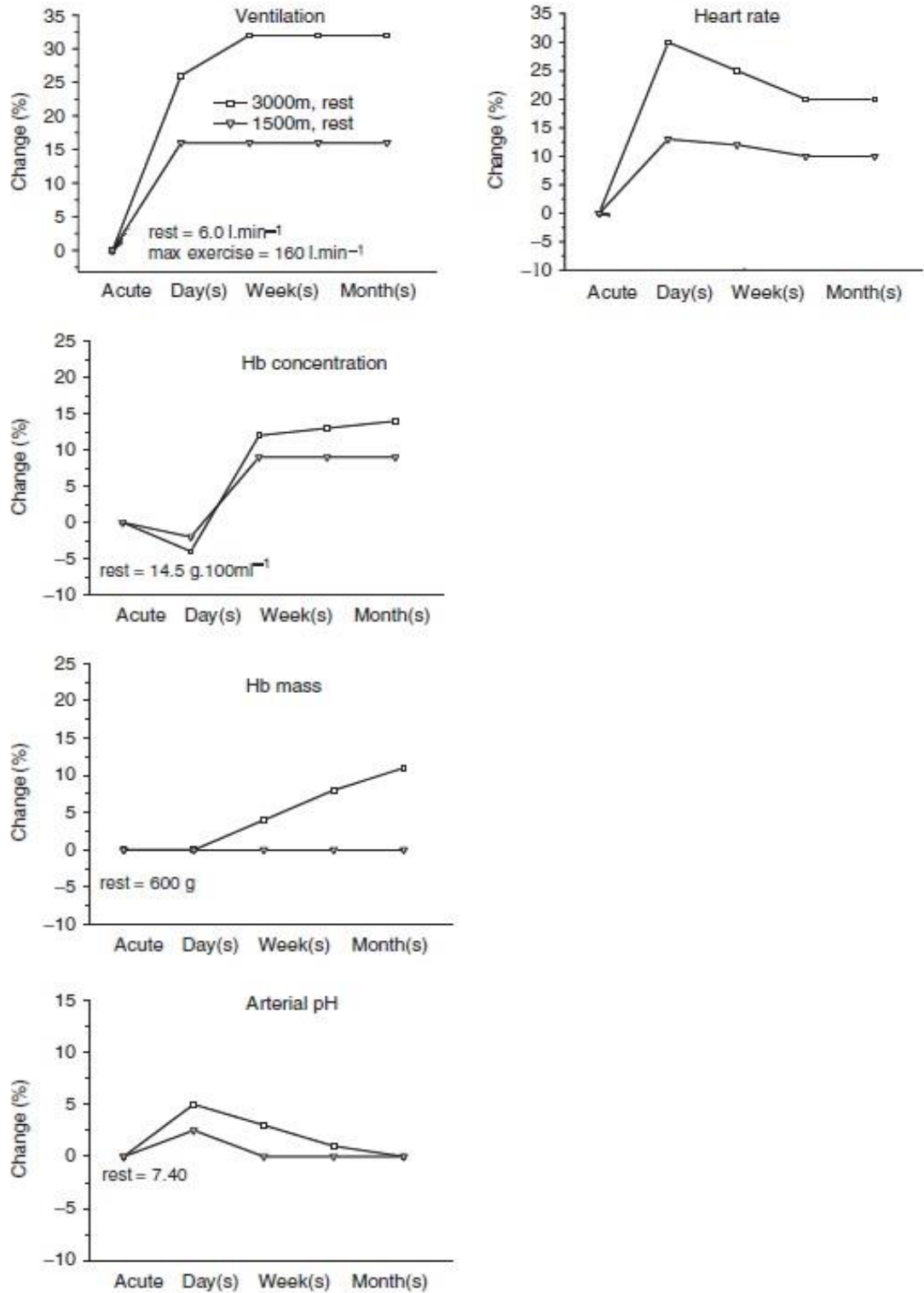


FIGURE 2.2: Schematic summary indicating the alteration of physiological variables at two altitudes (1,500 – 2,000 m and 3,000 – 3,500 m). (Bärtsch and Saltin 2008).

Notes: The x-axis denotes the time scale and the y-axis denotes relative changes using sea-level as baseline

Hypoxia has been seen to be an inducer of muscle adaptation through altitude exposure and with physical training. In regards to altitude exposure several muscle-related proteins involved in the transport of bicarbonate, hydrogen ions and lactate are upregulated as are red cell membrane proteins (Juel et al., 2003). These up-regulations augment the transport capacity of these ions, thus improving the maintenance of the acid-base balance at altitude. There is controversy however surrounding the subject of capillarisation at altitude with previous studies observing no increase in capillarisation when expressed as number of capillaries per fibre (Mizuno et al., 1990) whereas an increase in number of capillaries per unit of area has been reported especially at higher altitudes. Increases relative to the unit area of muscle has been reported as muscle fibre size is often reduced at altitude with reductions in cross sectional area of 20-30% above 4,000 m (Mizuno et al., 2008). Reduction in muscle size and the onset of this reduction appears to be correlated with increased altitude, i.e. the higher the altitude the sooner and more pronounced the hypertrophy is observed (Bärtsch and Saltin 2008). It appears however that muscle fibre size is not reduced below an altitude of 3,500 m and that muscle fibre types are usually unaffected by hypoxia (Bärtsch and Saltin 2008).

Knowledge of alterations in immunity at high altitude is not complete, however, this issue must be considered during the future investigation and implementation of exposure to hypoxia (with or without physical activity) as a therapeutic tool. Upon exposure to altitude an altered immune status is observed through increases in neutrophils and lymphocytes, cell proliferation and natural killer cells (Walsh and Oliver, 2016). A cell-mediated decrease in immunity during exercise at altitude supports the hypothesis that physical activity at altitude may pose a greater challenge to immune function than that at sea-level (Svendsen et al., 2016). Increased adrenaline, cortisol and interleukin-6 measures at rest during high altitude exposure (Mazzeo et al., 2001) support the hypothesis that physical activity at altitude may pose a greater challenge to immune function than that at sea-level.

SUMMARY

In summary in order to compensate for the altitude induced decreases in PaO₂ oxygen delivery is improved via changes in resting ventilation rate, circulating haemoglobin concentration and capillary density whilst metabolic remodelling at the tissues alters oxygen utilisation. Physiological responses upon exposure to environmental hypoxia are dependent on a number of factors including the severity of the hypoxia and the length of exposure. Both potentially positive and negative responses to altitude/hypoxia exist with regard to the use of this environmental extreme as a potential therapeutic tool either alone or in combination with physical activity.

2.5 SUBSTRATE OXIDATION AT ALTITUDE

OVERVIEW

Adenosine triphosphate (ATP) can be synthesised in the skeletal muscle in an oxygen dependent manner in the mitochondria via oxidative phosphorylation. This process occurs by utilising substrates

such as glycolytically derived pyruvate, fatty acids, amino acids and ketone bodies. Adenosine triphosphate can also be synthesised in an oxygen independent manner in the cytosol via glycolysis with the resulting pyruvate converted to lactate (Horscroft and Murray 2014). Under conditions of normoxia, (i.e. a plentiful oxygen supply), oxidative phosphorylation would normally meet the majority of the cells ATP requirements, due to a greater range of substrates available and a higher yield of ATP derived from glucose. Under conditions of environmental hypoxia however substrate partitioning is altered both acutely (Workman and Basset 2012) and chronically (Brooks et al., 1991).

Human acclimatisation to high altitude relies on mechanisms to sustain oxygen delivery to the tissues and on alterations to oxygen use with both mechanisms regulated, in part, by the hypoxic inducible factor (HIF) family of transcription factors which drive the expression of “hypoxia-sensitive” genes. Hypoxic inducible factor has recently being highlighted as an important mediator in the alterations in metabolism and EE induced by this environment (Palmer and Clegg 2014).

Definitive conclusions on both adaptive and metabolic responses to hypoxia are still debated and are affected by a number of confounding factors including physical activity, the length and extremity of the exposure, changes in environmental temperature and differences between low-landers exposed to altitude and populations residing permanently above sea-level. A seemingly major proponent of alterations in substrate use at altitude is the altitude induced changes in energy balance, in part due to changes in appetite and subsequent eating behaviour (Braun, 2008; Marriott and Carlson, 1996). As such it is often difficult to distinguish between the effects of hypoxia per se and the effects of a negative energy balance on substrate use particularly when working in an environment in which hypophagia and cachexia are commonly present. It is possible, if not likely, therefore that a number of the reported responses regarding substrate use at altitude are a combination of malnutrition with the addition of altitude rather than altitude alone (Brooks, 2014). The following section will outline the effect of exposure to environmental hypoxia on substrate use and highlight the influence of confounding factors such as physical activity on said changes.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

In a situation in which individuals are in a negative energy balance, due to altitude induced cachexia, the utilisation of the body’s fat stores is not uncommon (Marriott and Carlson, 1996). As such high altitude acclimatisation results that have indicated a sparing of muscle glycogen stores through a greater rate of fat utilisation are based upon elevated levels of FFA in the blood previously seen in both rats (Yin et al., 2009) and man (Jones et al., 1972), along with increased glycerol concentrations and a slower rate of muscle glycogen utilisation. Certain studies in which increases in the oxidation of lipids are seen (Louis and Punjabi 2009; Polotsky et al. 2003) have employed severe levels of hypoxia in their design (<10% O₂) or have used a repeated intermittent exposure protocol resulting in reduced glucose uptake or diminished glucose availability with subsequent increases in fat oxidation occurring

as a secondary outcome due to a cumulative effect of repeated exposures (Workman and Basset 2012).

Similarly it had previously been observed that following acclimatisation to altitude (4,300 m) an increased mobilisation and use of FFA occurred during exercise over carbohydrate (CHO) use in individuals who were experiencing loss of body mass and energy deficit (Young et al., 1982). These findings were also based primarily on the inferential measures of maintained levels of glycogen within the muscle and increased levels of TAG, glycerol and FFA following exercise at altitude. In a study from Barnholt et al. (2006) in which numerous parameters were measured in a group of calorie restricted individuals and in a well fed group during an altitude sojourn for 21 days at 4,300 m a dampening of the altitude induced increase in glucose availability was reported when calories were restricted. Lower blood glucose availability results in a fuel utilisation shift to sources such as fats and ketones in order to support energy needs. Such observations are similar to those reported during starvation (Saudek and Felig 1976 in Butterfield 1996) suggesting that these results may, at least in part, be due to calorie restriction rather than the effects of the altitude acclimatisation alone and that a major factor influencing substrate choice at altitude appears to be energy balance.

Accordingly a dampening of the often observed increase in blood glucose dependency at altitude is induced through calorie restriction (Young et al., 1982) as is a disruption in the acclimatisation process highlighted through dampened effects in insulin and EPO concentration compared to adequately fed individuals (Barnholt et al., 2006). Calorie restriction at altitude also appears to induce a blunted response of adrenaline availability which may acutely decrease CHO availability and use due to a direct action on muscle glycogenolysis and an indirect action on hepatic glucose production (Barnholt et al., 2006). Furthermore, increases in plasma FFA reported at altitude do not necessarily result in increases in their uptake as reported by (Roberts et al. 1996). Following acute exposure (<4 hours) to 4,300 m, a significant decrease in the uptake of FFA and in glycerol release by resting muscle was found despite increases in plasma FFA being reported. These findings were reported in conjunction with an increase in blood glucose dependence through an increased dependency on CHO during exposure.

Increases in the utilisation of carbohydrate derived fuel sources of glucose, glycogen and lactate are frequently reported when measured at altitude following acute (Brooks et al., 1991) and chronic exposure in men (Brooks et al., 1991; Roberts et al., 1996). Furthermore it has been suggested that when energy needs are met by diet there is little utilisation of lipid sources by working muscles at altitude. This phenomenon has been observed in a variety of experimental models including isolated muscle (Azevedo et al., 1995), the rat model (Cartee et al., 1991) and exercising dogs (Zinker et al., 1994). Exposure to altitude in female participants has also been examined in this regard culminating in the suggestion that differences exist between the sexes (Braun et al. 2000 Mawson et al. 2000). Braun et al. (2000) reported that at an altitude of 4,300 m, both acutely exposed and acclimated (10 days) females, exhibited no shift in utilisation of glucose at rest or during submaximal exercise. It is

suggested that a lower total CHO oxidation rate but similar glucose oxidation rate and therefore less glycogen utilisation is observed in women at altitude (Braun et al. 2000, Mawson et al. 2000, Brooks 2014). Mechanistic suggestions for the differences between males and females are discussed elsewhere in the thesis (sections 2.7, 2.10 and 6.5).

In a study in which feeding to meet energetic needs has been incorporated (Brooks et al., 1991) CHO, specifically glucose, was the main source of fuel at 4,300 m following acclimatisation at rest and during exercise. Accordingly a reduction in fatty acid consumption at the same altitude of 4,300 m following acclimatisation during both rest and exercise was observed in men fed sufficiently to cover need coupled with an increased glucose utilisation during exercise (Roberts et al., 1996). Reduced plasma glucose concentration following an acute exercise bout in hypoxia has also been observed when compared to a normoxic exercise bout (Bailey et al., 2015) demonstrated by area under the curve values ($10.1 \text{ mmol.L.hr}^{-1}$ in hypoxia v $10.5 \text{ mmol.L.hr}^{-1}$ in normoxia $p < 0.05$) as has an increase in RER during exercise and post-exercise recovery during an acute exercise bout at moderate altitude suggesting an increase in CHO utilisation (Figure 2.4) (Katayama et al., 2010). These findings support the preference for glucose derived fuels in hypoxia during both rest and exercise. Carbohydrate derived sources have therefore been proposed as the predominant fuel at altitude in those who are not in a negative energy balance and as more economical in an environment which is “oxygen poor” due to a greater yield of ATP per litre of oxygen consumption when compared to fat (McClelland, 2004).

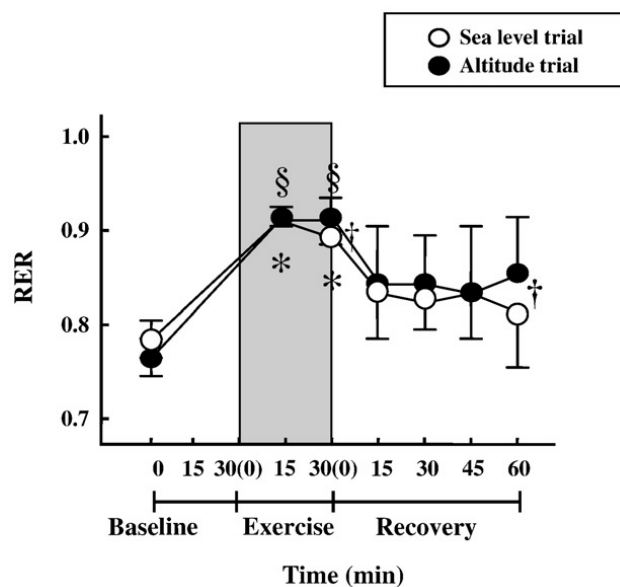


FIGURE 2.3: Respiratory exchange ratio (RER) during exercise and recovery at a simulated altitude of 2,000 m and at sea-level.

Notes: Values are presented as means \pm SE. * $p < 0.05$ vs baseline at sea level. $p < 0.05$ vs baseline at altitude. † $p < 0.05$ between moderate altitude and sea level (Katayama et al., 2010).

This “oxygen saving effect” of glucose utilisation based on fewer moles of oxygen required to obtain energy from glucose compared to obtaining energy from fat whilst in conditions where oxygen supplies are limited suggest that such a substrate shift would be beneficial. The oxidation of fatty acids requires more O₂ per ATP synthesised than the complete oxidation of CHO thus an increased reliance on carbohydrates may improve oxygen efficiency. This is despite a typical fatty acid providing 104 mol of ATP per mole of fuel compared to 31 mol of ATP for the complete oxidation of a mole of glucose (Brand, 1994). Although a greater amount of energy is yielded from a typical fatty acid, glucose is the most O₂-efficient fuel, eliciting between 6.0 and 6.3 mole of ATP per mole of O₂ oxidised compared with 5.6 mole of ATP per mole of fat (Lundby and Van Hall 2002). While increased use of glucose at altitude serves to maximise ATP yield per unit of oxygen, a suggested downside to this response is a more rapid depletion of limited carbohydrate stores meaning a “metabolic compromise” must be made at altitude, particularly when exercising, in order to deal with low O₂ availability and small carbohydrate stores simultaneously (Braun, 2008; McClelland et al., 1998).

Despite the general consensus of an increased dependence on CHO derived fuel sources in the adequately fed man at altitude a difference in the hypoxia induced effects of substrate use between males and females has previously been observed (Braun et al., 2000) suggesting that the generalisation of findings from men to the entire population may be inappropriate. The presence of Estrogen and Progesterone appears to direct substrate use toward increased fat and reduced carbohydrate use (Bunt, 1990) an effect which may be accentuated during the midluteal phase of the menstrual cycle when an elevation in Estrogen and particularly Progesterone occurs (Bunt, 1990). Accordingly and specific to altitude exposure work from McClelland et al. (1998) observed high altitude acclimated female rats showed no change in the proportion of energy derived from glucose or glycogen compared to rats at sea-level and that any shifts in substrate use were attributable to changes in exercise intensity. In agreement a study from Braun et al. (2000) reported that at an altitude of 4,300 m both acutely exposed and acclimated (10 days) females showed no shift to a greater utilisation of glucose at rest or during submaximal exercise. In fact blood glucose utilisation rates were lower at rest after 10 days exposure to 4,300 m than those observed at sea-level. Importantly these findings were reported in conjunction with no change in body mass suggesting that true sex differences exist with regard to substrate use at altitude and that these findings were not altered by a negative energy balance.

Mechanistic suggestions for the observed differences between males and females include the stimulation of the production of catecholamines and cortisol by physiological stresses such as hypoxia which provoke a shift away from CHO use and a greater reliance on fatty acids in females (Braun et al., 2000). As mentioned changes in ovarian hormones are also likely to have direct and indirect effects on carbohydrate and lipid metabolism (Bunt, 1990). A greater need to conserve limited glycogen stores at altitude may also be present in females compared to males leading to the suggestion that

the conservation of carbohydrate stores may override a shift towards greater utilisation of glucose at altitude (Braun et al., 2000; McClelland et al., 1998).

These findings are in opposition to the majority of findings conducted on males as previously discussed and as such the evidence leads to the suggestion that unlike males, females do not appear to shift substrate use to greater CHO dependency in response to hypoxia. Such findings must be considered in both scientific and field environments to ensure that sex differences do not impair research findings and that these differences are considered for any dietary and/or practical guidelines for individuals exposed to hypoxia.

There have been a number of suggested mechanisms for an increase in glucose uptake at altitude. Figure 2.5, adapted from Braun (2008), illustrates the known and postulated effects of acute exposure and acclimatisation on substrate use and metabolic economy. Skeletal muscle is a major site for postprandial glucose disposal and has been suggested as the most important tissue for glucose uptake in the human body (Heinonen et al., 2012) which may in part be responsible for the witnessed Increase in CHO oxidation, particularly during exercise at altitude (Braun, 2008). Normally the uptake of glucose is controlled by insulin, however this differs with exercise during which an increase in intracellular free calcium, which also induces the translocation of GLUT 4 transporters to the cell membrane, occurs to facilitate glucose entry into the cells of the muscle (Rose and Richter 2005). A hypoxia induced stimulation of GLUT4 via signalling pathways has been suggested as a possible mediator for glucose transport at altitude (Cartee et al., 1991) as has the intracellular enzyme AMPK that promotes blood glucose transport with stimulation such as from hypoxia or exercise (Fujii et al., 2006).

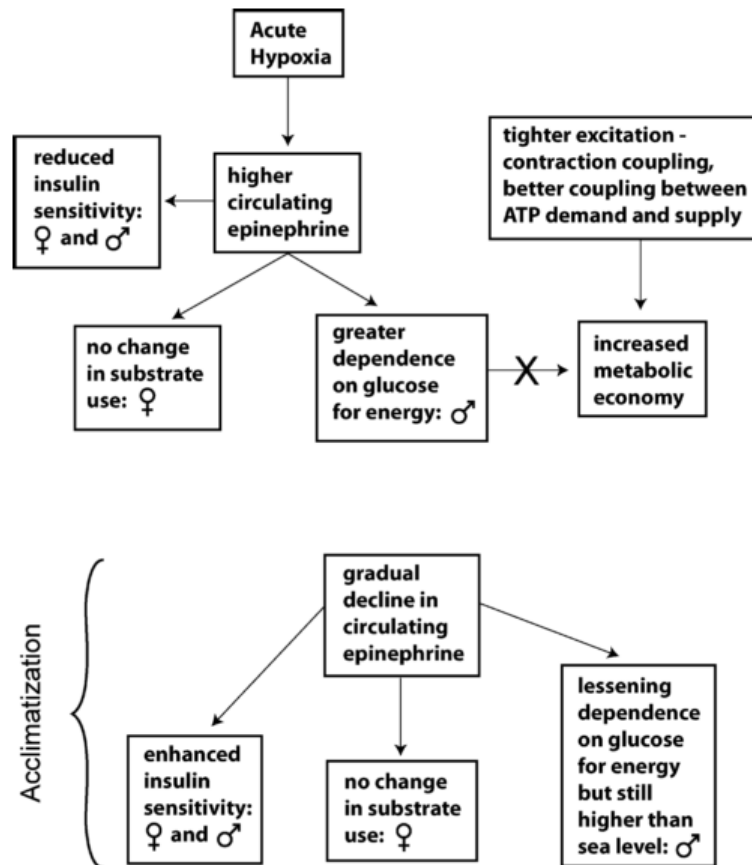


FIGURE 2.4: Known and postulated connections between acute hypoxia, acclimatisation, substrate use and metabolic economy (Braun, 2008).

Another signalling mechanism that mediates increased glucose uptake under hypoxia may be adenosine which is formed from the degradation of extracellular ATP during muscular contractions (Leuenberger et al. 1999; Bryan and Marshall 1999). Adenosine is also formed intracellularly during hypoxic or ischemic conditions and has been shown to potentiate and regulate both insulin and contraction-induced glucose uptake (Derave and Hespel 1999). Furthermore the removal of adenosine has been seen to decrease glucose transport in both situations (Han et al., 1998). In a study from Heinonen et al. (2012) a threefold increase in blood glucose disposal was observed in exercising muscle at an acute hypoxia corresponding to 3,400 m compared to normoxia. Interestingly exogenous adenosine increased muscle glucose uptake at rest although the inhibition of endogenous adenosine did not affect glucose uptake during exercise which suggests the importance of adenosine for regulating glucose uptake is present only at relatively high concentrations (Heinonen et al., 2012). An indirect role of adenosine is suggested to play a role in glucose metabolism by limiting lipolysis during exercise (Heinonen et al., 2012). Severe hypoxia has also been shown to stimulate the translocation of glucose across the plasma membrane (Cartee et al., 1991; Fujii et al., 2006).

Through previous work in cultured cells it has been suggested that the transcription factor, hypoxia-inducible factor 1-alpha (HIF α) is upregulated on exposure to altitude (Semenza et al., 1994) thus

increasing glycolysis and thereby attenuating oxygen utilisation and ATP synthesis (Wheaton and Chandel 2011). Thus an up-regulation of the transcription factor HIF leads to a greater dependency on glucose uptake as an adaptive response to altitude when oxygen availability is limited. Interestingly however mixed results within the previous literature suggest that biomarkers of beta-oxidation are not seen to be attenuated any more than biomarkers of oxidative phosphorylation as may be expected if a hypoxic induced shift towards CHO occurred (Horscroft and Murray 2014). Roberts et al., (1996) observed an increased glucose uptake and decreased fatty acid oxidation in human vastus lateralis muscle following 21 days at an altitude of 4,300 m however it is unclear whether this increase in glucose uptake supported increased lactate production through lactate dehydrogenase or pyruvate oxidation via pyruvate dehydrogenase and the Krebs cycle (Horscroft and Murray 2014).

A loss of cellular mitochondrial content is suggested to be induced by the down regulation of mitochondrial biogenesis factors such as peroxisome proliferator-activated receptor γ co-activator 1 alpha or beta at altitude alongside the up-regulation of mitochondrial autophagy factors (Horscroft and Murray 2014). This adaptation coupled with the up-regulation of pyruvate dehydrogenase kinase (PDK) isoforms deactivates pyruvate dehydrogenase, which impairs pyruvate entry into the tricarboxylic acid cycle (TCA cycle)/ Krebs cycle culminating in a high rate of glycolysis relative to oxidative phosphorylation (Kim et al., 2006; Papandreou et al., 2006). Despite this mechanistic work in cultured cells more debate exists regarding the effects of hypoxia on energy metabolism in mammalian tissues *in vivo*.

More precisely HIF-1 is a heterodimeric protein that is composed of an O₂-regulated HIF-1 α subunit and a HIF-1 β subunit. The levels of HIF-1 α protein activity increase initially at altitudes below 5,000 m before declining to steady state level whereas a persistent increase in HIF activity occurs at altitudes above 5,000 m (Palmer and Clegg 2014). At the cellular level HIF signalling promotes development of a glycolytic phenotype. The two suggested mechanisms for this include the increased expression of pyruvate dehydrogenase kinase 1 which contributes to increased glucose use and increased glucose uptake in most tissues. hypoxic inducible factor has the effect of down-regulating mitochondrial respiration thus at the mitochondrial level HIF has the effect of moderating glycolytic flux to the Krebs cycle thereby giving rise to lactate production. Secondly HIF signalling acts to affect the expression of muscle type lactate dehydrogenase isoforms (Brooks, 2014). Hence on exposure to altitude the predisposition to direct glycolytic flux to lactate production rather than acetyl-co A formation and disposal via the Krebs cycle is encouraged. The HIF-supported increase in lactate production is an advantage because lactate is a preferred fuel in working muscle during hypoxia and the main gluconeogenic precursors therefore lactate produced in one cell is a useful substrate in another cell (Brooks, 2014). Other effects witnessed at altitude that the activation of HIF upon exposure is said to contribute include an initial increases in basal metabolic rate, a reduction in appetite and an increase in circulating Leptin culminating in loss of body mass (Palmer and Clegg 2014) (as discussed elsewhere in this review of literature).

More recent work regarding the use of acute exposure on subsequent substrate use in both hypoxia (Morishima and Goto 2014; Katayama et al. 2010) and normoxia (Workman and Basset 2012) have added to the existing data. Workman and Bassett reported an increase in the utilisation of fat sources immediately after acute exposure to hypoxia whilst resting in normoxia (Workman and Basset 2012). These findings are suggested to be as a result of the repayment of an oxygen deficit induced by reduced FiO_2 through increased autonomic neuroendocrine stimulation of lipolysis, increased catecholamine release partially responsible for stimulating higher lipid usage, and as a response to greater CHO utilisation during exposure to hypoxia (Workman and Bassett 2012). Alternatively when resting in hypoxia following an acute exercise bout Katayama et al. (2009) demonstrated increased utilisation of CHO suggesting that the environment in which the “post” measure occurs may alter the preferred substrate use.

Although a shift in substrate use occurs under the conditions of hypoxia there appears to be no reduction in the delivery of glucose or FFA at altitude due to the hypoxic induced increases in cardiac output and tissue perfusion. As blood flow increases to maintain arterial O_2 delivery it also increases the delivery of all substrates including glucose meaning that within the initial hours at altitude arterial glucose is unaffected and only slightly decreases (5 - 7%) after chronic exposure (Roberts et al., 1996).

Given the data available indicating suppression of glycerol release and FFA uptake from working limbs at altitude it may be appropriate to conclude that intramuscular lipolysis as well as mitochondrial uptake of activated FFAs uptake is suppressed by acclimatisation to altitude in adequately nourished humans. This conclusion is based on the absence of glycerol release from the muscle. As skeletal muscle and adipose tissue lack the enzyme glycerol kinase, we can interpret the absence of glycerol release from muscle after acclimatisation to mean suppression of lipolysis within the muscle. This absence of glycerol release from working muscle following acclimatisation to altitude also means it can be suggested that feed forward regulation of glycolysis results in mitochondrial acetyl-coA formation and down-regulation of mitochondrial FFA uptake (Brooks, 2014).

Skeletal muscle due to its relatively high capacity for respiration is of interest due to its metabolic rate being altered acutely through exertion, substrate preference and mitochondrial density. Chronic alterations of skeletal muscle occur through training, diet and environmental factors. A conclusive review from Horscroft and Murray (2014) observed that a loss of mitochondrial density in human vastus lateralis muscles seems to occur after long term exposure to hypoxia (Levett et al., 2015) but not after short term exposure (Jacobs et al., 2013). Furthermore it has also been seen that the skeletal muscle of highland Tibetans tends to be less rich in mitochondria when compared to lowlanders (Kayser et al., 1996) supporting the notion that loss of mitochondria density is an adaptive trait at altitude. The review also concluded that the attenuation of oxidative processes such as beta-oxidation, the Krebs cycle and oxidative phosphorylation also seems to be induced by environmental hypoxia. Moreover it is suggested that this attenuation may be secondary to the aforementioned reduced

mitochondrial density in the short term (<14 days) as these reductions have been witnessed without any change in mitochondrial density (Horscroft and Murray 2014). Reductions in mitochondrial density have been seen in some medium term (≤ 42 days) and most long term (>42 days) exposures. It is therefore suggested that the hypoxia induced remodelling of mitochondrial pathways occurs before any loss in mitochondrial density at altitude. The effect of hypoxia on glycolytic capacity however is less clear.

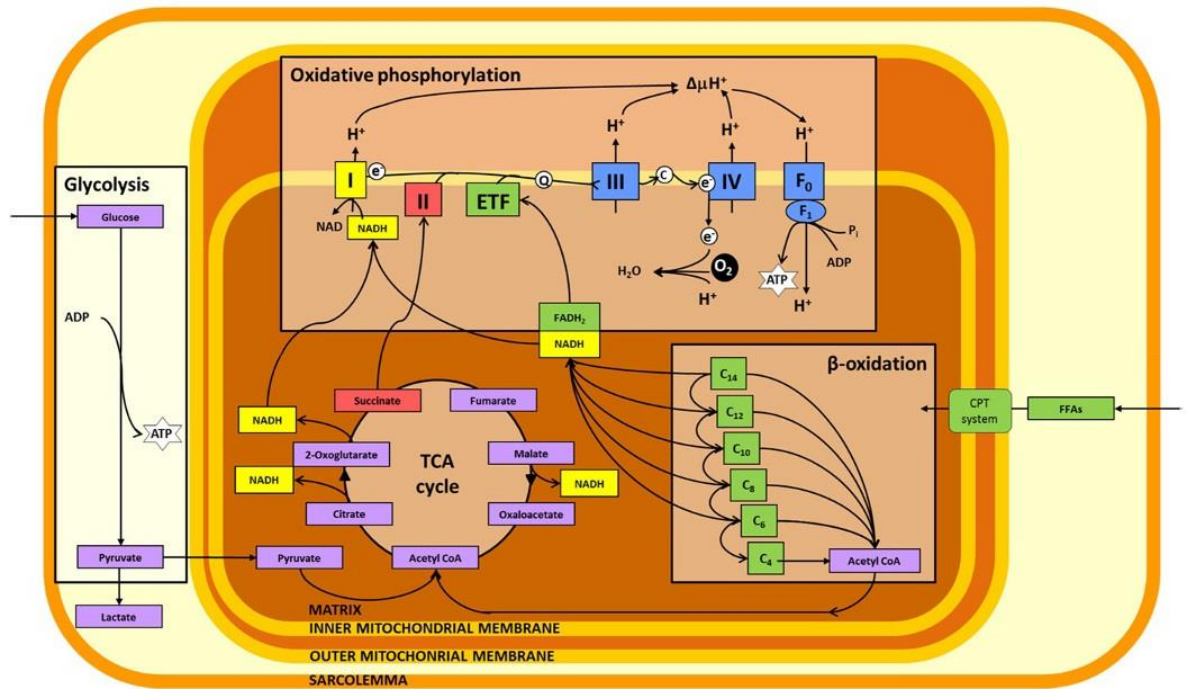


FIGURE 2.5: Energy metabolism in the skeletal muscle (Horscroft and Murray 2014).

Notes: Adenosine diphosphate (ADP), Adenosine triphosphate (ATP), Flavin adenine dinucleotide (FADH), Nicotinamide adenine dinucleotide (NADH), Electron-transferring flavoprotein (ETF).

Another factor of relevance at altitude is diuresis. Diuresis at altitude is frequently reported (Boyer and Blume 1984). Diuresis serves to concentrate haemoglobin and thus improve the peripheral delivery of oxygen during the initial hours at altitude. This diuresis combined with a shift in circulating levels of fuel metabolites and increased metabolic need in sojourners suggested that altitude exposure causes changes in energy substrate utilisation (Boyer and Blume 1984) as previously suggested.

Previously studies have reported that glucose administration during prolonged physical activity is beneficial (Fulco et al., 2005) highlighting CHO derived fuels as the preferred option at altitude. Elevated adrenaline levels in the blood, stimulates glycolysis and increases the availability of pyruvate for either further oxidation in the mitochondria or for lactate production (Roberts et al., 1996). Previous increases in CHO utilisation during exercise have correlated with increased adrenaline concentrations (Roberts et al., 1996).

It is well established that at sea-level with increasing exercise intensity an increase in the contribution of CHO to total energy generation occurs. It has also been established that a given work rate performed at sea-level will be relatively higher when performed at high altitude. Based on these established findings (Lundby and Van Hall 2002) it is suggested that the reason for concluding that CHO use increases at altitude during exercise is due to experimental design as the same relative intensity exercise in both conditions is not often compared. In an attempt to address this point Lundby and Van Hall examined substrate utilisation during exercise at the same absolute and relative exercise intensity and concluded that submaximal substrate utilisation is unchanged with acute and chronic hypoxia when the work rate is adjusted to match relative sea-level intensity and that in agreement with previous research (Roberts et al., 1996) CHO utilisation increased at altitude compared to sea level when absolute exercise intensity is compared. It was therefore concluded that previous increases in CHO utilisation at high altitude might be caused by the increase in relative exercise intensity. It is unknown the extent to which the increase in CHO utilisation seen at altitude is a change in plasma substrate utilisation or a shift on the intramuscular level.

Acute exposure to altitude exaggerates the blood lactate response to exercise for a given workload during acclimatisation. After prolonged exposure however maximal lactate concentration is reduced and submaximal lactate levels tend to be or are also reduced (Bärtsch and Saltin 2008). It has been suggested that the obvious candidate for increased rate of appearance for lactate during exercise is the liver. Enhanced hepatic release and muscle uptake has previously been recorded in exercising dogs at hypoxia (Zinker et al., 1994) but has not been observed in humans (Ahlborg and Felig 1982).

In support of an increased use of glucose at altitude and the potential beneficial effects of training under moderate hypoxia it was observed that four weeks of moderate intensity (55% of $\dot{V}O_2$ max three times a week) endurance training (55% of $\dot{V}O_2$ max three times a week) under hypoxic conditions (15% O_2) resulted in a greater improvement in postprandial hyperglycaemia compared to training in normoxic conditions displayed by area under the curve values (Morishima et al. 2014). Similar findings from Kelly et al. (2010) in healthy adults were described as the plasma glucose response to a 75g glucose load was significantly attenuated under a simulated altitude of 4,300m compared to normoxia suggesting hypoxia may have a preventative effect on postprandial hyperglycaemia. Further work conducted by Morishima et al. found that over a period of 7.5 hours during which individuals rested and conducted three exercise bouts at moderate intensity a marked increase in CHO oxidation occurred compared to a trial in which individuals rested and exercised in normoxia (Morishima et al. 2014). However in this study neither rest alone nor rest and exercise combined in moderate hypoxia attenuated postprandial glucose responses despite hypoxia markedly promoting CHO oxidation. It must be considered therefore that substrate levels in the blood do not necessarily correspond correctly to substrate utilisation as substrate levels such as glucose and lactate are influenced by rates of appearance and disappearance (Morishima et al. 2014).

SUMMARY

In summary the relative contribution of glycolytic versus oxidative ATP production is increased by a hypoxic stimulus which may be exaggerated with exertion (Horscroft and Murray 2014). It is suggested based on the majority of the literature that muscle oxidative metabolism is lowered by exposure to environmental hypoxia which may precede a loss in muscle mitochondrial density however the total capacity for skeletal muscle glycolysis is not consistently altered by environmental hypoxia. Overall hypoxia induces attenuation of whole muscle fatty acid oxidation, whilst glucose uptake is maintained or increased in the face of down regulation of oxidative metabolism.

2.6 LOSS OF BODY MASS AND ALTERATIONS IN METABOLIC RATE AT ALTITUDE

OVERVIEW

Physiological responses to hypoxia in humans have been reported to alter energy balance and result in a loss of body mass (Kayser and Verges 2013; Barnholt et al. 2006). There are increases in the energy requirements of individuals and the subsequent breakdown of energy stores such as fat in both healthy normal weight (Westerterp, 2001) and obese individuals (Lippl et al., 2010). The magnitude of weight loss is also determined to some extent by the duration of the stay and the altitude attained (Marriott and Carlson, 1996) although acute exposures from 0 – 24 hours to altitudes greater than 10,000 feet/ 3,048 m have also been associated with anorexia (Brooks, 2014). These findings have led to the suggestion hypoxia has the ability to induce reductions in weight and fat mass in humans (Lippl et al., 2010).

In a recent study, a cross-sectional and nationally representative data set of 422,603 observations, representative of 207 million Americans, reported an inverse dose-response relationship between the elevation at which an individual resides and the prevalence of obesity (Voss et al., 2013). Based on life time exposures prevalence of obesity decreased with increased elevation by 200m increments with a wider variance of BMI at lower elevations. Furthermore, the demonstration of a 7.5% higher prevalence of obesity in Americans living at sea-level compared to those residing at 1,000 m was observed. This study suggests a role of residing at altitude in the long term homeostasis of weight which is of importance given that the majority of previous investigations have highlighted the short term effects alone.

Since the observed decreases in body mass of mountaineers travelling to and spending prolonged periods at altitude, the cachexic effect of such an environment has been considered. Previously, confounding factors often experienced at altitude have made it difficult to elucidate the independent effect of hypoxia on such changes and to date debate exists regarding the underlying mechanisms of weight loss and the changes in body composition at altitude. Such confounding factors include physical exertion, changes in food types, availability of food, malnutrition, and extreme temperatures amongst others. Exposure to altitude is often experienced together with exposure to cold conditions.

Figure 2.7 summarises these effects of exposure to both altitude and the cold on energy and fluid balance.

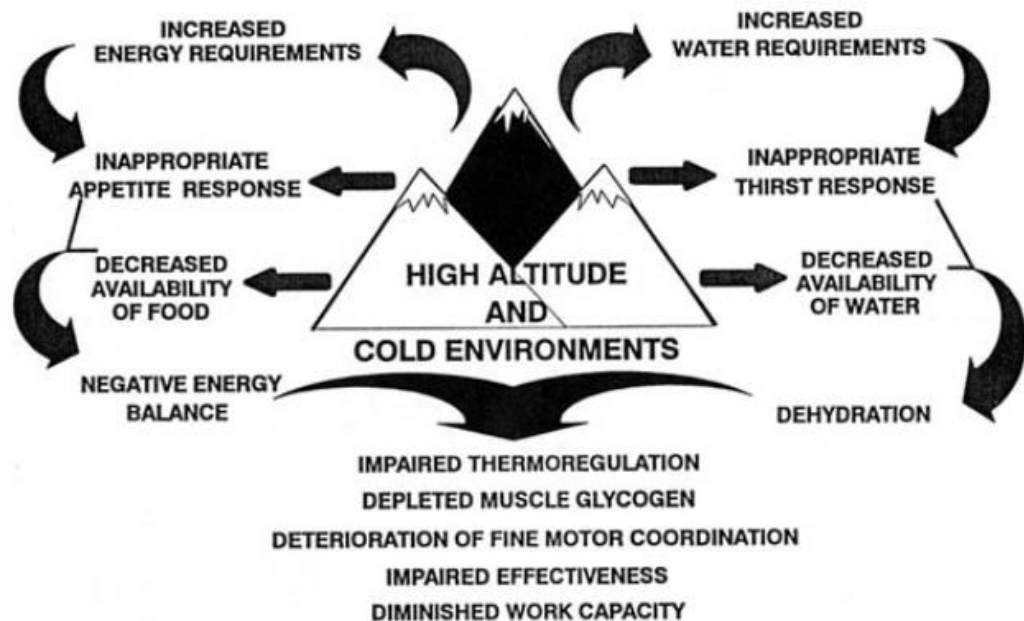


FIGURE 2.6: Representation of the influence of cold and high-altitude environments on energy and fluid balances and resulting physiological consequences (Askew, 1995).

A growing body of literature regarding the physiological mechanisms of body mass loss independent of confounding factors now exists however. As such the effect of hypoxia on metabolic rate, subsequent changes in body mass and accompanying alterations in body composition are reviewed in the following section. The current section will also review the effects of environmental hypoxia on changes in metabolic rate and body mass in view of differing states of energy balance that are often demonstrated upon exposure to altitude and review previous work investigating the use of dietary interventions at altitude. Furthermore, the physiological responses to intermittent hypoxia will be highlighted and a Table of the most relevant published research is presented.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

Exposure to high and extreme altitudes (5,300 m – 8,848 m), has been seen to elicit a steady reduction in weight with increasing severity of hypoxic exposure, the higher the altitude the greater the weight loss (Reynolds et al., 1999). Although the exact body composition changes at altitude are contradictory some previous research (Reynolds et al., 1999; Westerterp, 2001) has attributed a major part of losses in weight to reductions in fat mass. In a recent review with regard to sustained hypobaric hypoxia in both the laboratory and field environment body weight losses of 5 – 15% (Rose et al. 1988; Tanner and Stager 1998; Westerterp-Plantenga et al. 1999; Reynolds et al. 1999) have recently been identified (Wing-Gaia, 2014). Mean losses of 150 -200g/day has been outlined as typical whilst at high altitude

(Marriott and Carlson, 1996) with some suggestion that the greatest losses of mass occur within the initial period of exposure (five days) (Mawson et al., 2000) however this remains debated. Although it is unlikely loss of body mass can be completely abated, there is some suggestion that below 5,000 m losses of body mass can be reduced through prescribed energy intake and physical activity based upon altitude induced increases in basal metabolic rate (Butterfield et al., 1992; Consolazio et al., 1972; Kayser et al., 1993). The issue of increased basal energy needs coupled with frequently reported reduced calorie intake (discussed in detail in upcoming sections) whilst individuals are exposed to high altitude has been suggested to result in a potential energy deficit of approximately 500 kcal.day⁻¹ (Butterfield et al., 1992; Marriott and Carlson, 1996). Such a deficit would result in an approximate loss of 0.5 kg of fat mass per week although a number of studies have observed greater losses than that expected from basal energy needs alone (Krzywicki et al. 1969; Boyer and Blume 1984; Consolazio et al. 1972; Rose et al. 1988; Butterfield et al. 1992) suggesting other considerations including the adequacy of energy intake at altitude contribute to the extent of such alterations. Furthermore in studies in which the doubly labelled water technique of measuring EE have been employed during an ascent of Mount Everest a requirement of 536 kcal.day⁻¹ for the acclimation to the increasing altitude alone has been reported (Pulfrey and Jones 1996; Reynolds et al. 1999).

SUMMARY

The exact mechanisms behind losses of body mass at altitude are largely uncertain and it is unknown if certain individuals only are affected or if there is a universal effect (Netzer et al., 2008). Previous literature however has outlined several factors that may contribute to energy deficits and loss of body mass at altitude including; loss of appetite and under nutrition, meaning a decreased food intake thus resulting in fewer calories consumed compared with those expended (Lippl et al., 2010; Voss et al., 2013). A more exhaustive review of the effects of altitude exposure on appetite is presented in a later section of this review of literature.

2.7 THE EFFECTS OF ALTITUDE ON METABOLIC RATE AND BASAL ENERGY NEEDS

OVERVIEW

Oxygen supply is known to be one of the various ambient conditions that have an influencing effect on resting EE (Oltmanns et al., 2006). An elevation in basal metabolic rate, likely due to sympathetic activation (Butterfield et al., 1992) and an increase in EE in line with an increase in relative effort at a given intensity compared to sea-level (Henderson et al., 2007) is commonly observed. From work carried out on Pikes Peak (Butterfield et al., 1992; Mawson et al., 2000) it was observed in the first two days at an altitude of 4,300 m basal energy needs of men were elevated by as much as 40% above sea-level values before declining over a period of three to four days to stabilise at approximately 17% above sea-level values. At high altitude the elevation in EE has been likened to that of moderate to high intensity sea-level exercise (Westerterp and Klass 1994; Wing-Gaia 2014). Accordingly in

individuals climbing Mount Everest EE values of 1.85 – 3.0 times greater than sea-level resting measures were recorded through doubly labelled water techniques (Westerterp et al. 1985; Westerterp and Klass 1994; Butterfield et al. 1992; Reynolds et al. 1999). Butterfield (1992) showed that an extra 500 kcal.day⁻¹ was required for men at 4,300 m in order to maintain body weight, and perhaps more at altitudes above 5,500 m in part due to an approximate increase of 300 kcal.day⁻¹ in basal energy needs. Over time these deficits have the potential to result in significant changes in body mass and composition. Interestingly the energy deficit for women has been observed at less than half of that for men consistent with the ability of women to maintain metabolic homeostasis (Mawson et al., 2000). Increases in basal oxygen consumption in women have been seen to increase upon exposure to altitude (Mawson et al., 2000). The increases in glucose flux in women during exercise however occur to a smaller degree at altitude being little different from the changes experienced at sea-level (Brooks, 2014), particularly if relative intensity is considered (Braun et al., 2000). Overall a lower total CHO oxidation rate but similar glucose oxidation rate and therefore less glycogen utilisation is observed in women at altitude.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

Weight loss induced through caloric restriction is generally made up of water, fat and FFM. At altitude it is suggested that losses of fat free mass approximately comprise between 60 – 70% of total weight loss when induced through caloric restriction (Fulco et al., 2002; Rose et al., 1988; Wing-Gaia, 2014) compared to approximately 25% of weight loss at sea-level (Weinheimer et al., 2010). In individuals exposed to an extreme hypobaric environment equivalent to an altitude of 8,848 m 67% of losses derived from FFM were reported in conjunction with a 17% reduction in thigh cross-sectional area (Rose et al., 1988). Similar losses of muscle mass following exposure to altitudes above 5,000 m in the field are observed (Pulfrey and Jones 1996; Hoppeler 1990; Reynolds et al. 1999) highlighting the high altitude induced effect of “muscle wasting” in humans although it is less clear as to the reason for why such a large percentage of weight loss is derived from FFM. Accordingly it has been suggested that at moderate altitudes a greater proportion of fat mass contributes to losses of body mass (Boyer and Blume 1984).

As such the observed composition of weight loss at altitude is dependent on numerous factors including the technique of measure to determine such changes. Reported shifts in compartmental fluid as a response to high altitude exposure (Westerterp et al., 1996) has an effect on the results of indirect measures (Marriott and Carlson, 1996; Wing-Gaia, 2014). Altitude induced diuresis may also complicate findings of body composition in studies investigating the acute effects of exposure (Boyer and Blume 1984; Butterfield 1996). FFM is an important contributor in the regulation of metabolism as it contains glucogenic amino acids and nitrogen (Wing-Gaia, 2014). At high altitude loss of FFM has been seen to compromise muscle strength (Sergi et al. 2010), immune function (Murdoch, 1995), aerobic capacity (Hoppeler, 1990) and physical performance (Fulco et al., 1998) which can increase

the risk of injury and illness. Other studies have however reported greater reductions in fat mass compared to fat free mass (Armellini et al. 1997; Tanner and Stager 1998) although limitations regarding measurement technique and differences in the altitude attained complicate direct comparisons across studies.

It is suggested that altitude exposure may alter muscle protein synthesis or degradation with hypoxia being the largest contributor to observed muscle wasting at altitude (Wing-Gaia, 2014). Other suggestions include decreased physical activity, disruptions in sleep cycles, exposure to cold temperatures and nutrition related changes in protein metabolism (Wing-Gaia, 2014). Studies in rodents have observed decreases in muscle protein synthesis following as little as six hours of exposure to hypobaric hypoxia (Preedy et al., 1985). A reduction in expression of the signalling pathway mTOR following seven to nine days of exposure to a hypobaric environment (4,559 m) has previously been observed in humans (Brugarolas et al., 2004). Also in humans a blunted muscle protein synthesis response was observed following a bout of resistance exercise in individuals after they had been exposed to normobaric hypoxia for 3.5 hours compared to the same resistance exercise following normoxic rest (Etheridge et al., 2011). A three-fold increase in muscle protein synthesis was observed when individuals were free from the stimulus of hypoxia in comparison to a 1.4 fold increase when individuals had been exposed suggesting that hypoxia plays a role in inhibiting the normal stimulation of protein synthesis. Training at altitude and increased energy intake has however been suggested to have a protective effect on protein utilisation (Worme et al., 1991). Such findings originate from a study in which energy intake at altitude ($3430 \pm 79 \text{ kcal.day}^{-1}$) was increased compared to sea-level intake ($2354 \pm 71 \text{ kcal.day}^{-1}$), achieved through a high carbohydrate military ration, coupled with rigorous physical activity for 31 days resulted in the maintenance of physical performance and lean body mass (Worme et al., 1991).

2.7.1 EFFECT OF ENERGY BALANCE

Energy intake alone is unlikely to be able to completely mitigate losses of muscle mass upon exposure to high altitude. This suggestion is based upon the observed altitude induced downregulation of mTOR (Brugarolas et al., 2004) being independent of hypophagia (Favier et al., 2010). It has been observed however that whether one is in a positive or negative energy balance can have an influence on muscle protein synthesis. In the absence of sufficient energy; whole body proteolysis is increased as amino acids are oxidised for energy or gluconeogenesis. An inadequate energy intake is therefore likely to contribute to an impairment of muscle protein synthesis and an increase in proteolysis at altitude which is induced and exacerbated by the altitude induced reduction in energy intake (Favier et al., 2010). Although a hypoxia-induced downregulation of muscle protein synthesis occurs regardless of energy balance greater deficits in caloric and protein intake may worsen the problem. Therefore targeting dietary intake whilst at altitude may somewhat alleviate the negative effects of altitude on protein synthesis and thus loss of FFM. Further work regarding the practicality of high calorie and protein diets is required however, particularly at higher altitudes where palatability of food is

compromised. The following section will address studies in which specific diets have been employed at altitude.

As stated, at higher altitudes (> 5,500m) loss of muscle mass accounts for a greater proportion of overall weight loss (66 – 73%) than loss of fat mass (Murray and Montgomery 2014; Rose et al. 1988; Boyer and Blume 1984). This is perhaps as a result of evolutionary effects of hypoxia on protein synthesis it is suggested (Murray and Montgomery 2014). These effects may be mediated through inhibition of gene transcription and translation (Liu and Simon 2004) and through the inhibition of protein synthesis and promotion of muscle catabolism through the induction of the metabolic sensor AMP kinase (Sanchez et al., 2012).

As previously discussed in this review of literature in individuals in which daily dietary energy intake is adequate; working muscle runs on CHO-derived fuels whilst the rest of the body largely utilises fat for energy. Furthermore during “non-exercise” parts of the day the whole body depends on the catabolism of lipid and protein stores to support energy substrate supply hence losses of body mass can occur at altitude (Brooks, 2014). In previous studies in which food intake has been documented a reduction of approximately 200 kcal.day⁻¹ in men during exposure to 4,300m is observed (Butterfield et al. 1992; Butterfield 1996; Brooks 2014). In the aforementioned studies by Butterfield et al., (1992) and Mawson et al., (2000) however the maintenance of energy and nutrient balance through planned eating was possible. The data provided by Butterfield et al., (1992) using nitrogen balance, which is considered the most sensitive measure of lean body mass and energy balance, (Calloway, 1975) is unique work in the field. These findings have led to the suggestion that losses of body mass at altitude is a consequence of, in part, reduced energy intake and could thus be abated by sufficient intake to match the increased need at altitude (Butterfield et al., 1992; Consolazio et al., 1972; Kayser et al., 1993).

It has been suggested however that these findings lack relevance to field activity due to either enforced feeding (Butterfield et al., 1992) or unrealistic situations including “comfortable conditions” and a large range of palatable foods (Kayser et al., 1993). Furthermore loss of weight was not prevented in studies from Butterfield et al. (1992) and Consolazio et al. (1972) and it is unclear if the prevention of functionally important body mass loss occurred (Macdonald et al., 2009). In a study from Macdonald et al. (2009), which reflected a typical expedition, a decrease in body mass of 110g/d was observed, of which fat mass (45%), total body water (35%) and residual mass (protein and glycogen) (25%) all contributed following the 21 day altitude stay during which an increased energy intake through carbohydrate supplementation was employed. The increased energy intake of between 10,000 – 15,000 kcal through the consumption of CHO drinks (which met the increased estimated energy demands induced by the hypoxia per se in more than half of the participants over 21 days) failed to prevent loss of both fat and fat-free mass in individuals at an altitude ranging from 900m to 5,400m. The dietary intervention was provided ad libitum in order to reflect field conditions

and was reported as palatable and to cause no gastrointestinal distress. These findings show that increased energy intake through a palatable and realistic field based carbohydrate intervention does not preserve functional residual body mass and suggest against a primary role of negative energy balance for changes in body composition at altitude and propose other contributory factors are present. Interestingly a higher initial fat mass had no protective effect of loss of residually important body mass during the sojourn despite previous studies highlighting fat as a substantial energy store (Reynolds et al., 1999).

Similarly studies that have employed dietary manipulations consisting of differing macronutrient contributions at altitude have resulted in failure to prevent loss of body mass. As a consequence of reduced energy intake at altitude, protein intake is also compromised (Wing-Gaia, 2014). As such the use of supplemental protein has been investigated. Work in which protein supplementation of 5.76, 2.88 and 2.88 g per day of leucine, isoleucine and valine, respectively was employed during a 21 day trek at a mean altitude of 3,225m observed significant losses of body and fat mass in both a control and supplementation group (Skena et al. 1992). These findings were found in conjunction with an overall 4% reduction in energy intake suggesting that this method was unsuccessful in both maintaining sea-level energy intake and in abating loss of body mass. However in comparison to the control in the same investigation an increase in lean mass and a prevention in the reduction of arm muscle cross sectional area suggest some potential of protein supplementation in the abating of muscle mass loss at hypobaric hypoxia (Skena et al. 1992). Decrements in performance measures at a moderate altitude of between 2,500 – 3,800m (Bigard et al., 1993) has been reported in other work in which protein supplementation has been employed has however reported. A supplemented ingestion of $2.5\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ resulted in an almost 27% decrease in endurance time at 50% maximal voluntary contraction exercise compared to a lower ingestion of $1.5\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$. Such findings highlight possible considerations and limitations to nutrient supplementation at altitude and the need for further work. Although logical in an attempt to retain FFM at high altitude increased protein intake has a number of possible limitations in the field. Such limitations include the feasibility and practicality of consuming up to $1.6\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ which has been outlined as the required amount to support FFM retention during negative energy balance (Pasiakos et al., 2013). Secondly with regard to the thermogenic effects of protein and an energy cost of 20 – 30% (Tappy, 1996), it has been suggested to mean that in an environment in which oxygen supply is limited increasing protein intake may be too “costly” in an energetic sense (Wing-Gaia, 2014). The protein induced effect on satiety is also considered a possible limiting factor for proposed increased intakes at altitude. Although considered beneficial in certain circumstances, additional protein at high altitude may increase the commonly reported blunted appetite response (Westerterp and Kayser 2006) and thus result in reduced overall energy intake. The implications of such may be of particular importance if increased protein intake results in decreased intake of carbohydrate culminating in impairment in performance and a reduction in glycogen stores (Bigard et al., 1993).

As already discussed sustained exposure to hypoxic conditions as a consequence of exposure to high altitude or critical illness induces cellular hypoxia thus threatening cell function and survival. Established features of the hypoxia response include measures to maintain blood oxygen content, through erythropoiesis, and to reduce tissue oxygen demand. The latter achieved through inhibition of mitochondrial oxidative phosphorylation and a possible loss of mitochondrial density. It has been hypothesised by Murray and Montgomery (2014) that altitude induced cachexia is a protective evolutionary adaptation rather than simply being a pathophysiological dysregulation. It is suggested that the catabolic response to altitude is orchestrated and is metabolically advantageous and protective under hypoxic conditions. Specifically, altitude induced cachexia may offer an advantage for survival when disease states are complicated by reduced cellular oxygen delivery and it is this “wasting” effect that has been selected for. Reduction of muscle fibre size will reduce the diffusion distance for oxygen and substrates, meaning that cachexia will provide a beneficiary adaptation.

Synthesis of ketone bodies as a result of lipolysis and the release of amino acids due to muscle break down are suggested mechanisms to offer hypoxic protection. More specifically protein breakdown releases amino acids with some of these able to undergo direct oxidative deamination to their corresponding ketoacids in the liver yielding reduced NAD (NADH) and a free ammonium ion which is converted to urea and excreted. Most amino acids however are initially transaminated rather than deaminated in this situation. Following this, transamination occurs. The resulting ketoacids from this process (the carbon skeleton of the deaminated amino acids) enter pathways that essentially fuel the Krebs cycle. Around 80% of the mitochondrial oxygen consumption is estimated to be coupled to ATP synthesis at rest of which 25-30% is accounted for by protein synthesis. It is suggested that a reduction in protein synthesis, which is seen during the altitude induced catabolic state, may therefore be advantageous during situations when oxygen availability is scarce (Murray and Montgomery 2014). Adenosine triphosphate demand for protein synthesis can fall by 93% in some animals under hypoxic conditions (Hochachka and Lutz 2001) although in human studies it has been suggested that there is no resting effect on synthesis, only on the stimulated aspect, when ATP demand increases (Etheridge et al., 2011). Nevertheless it is suggested that ketone bodies synthesised as a result of lipolysis and amino acids released by muscle breakdown act not only as metabolic fuels but also as metabolic modulators offering hypoxic protection. Specifically ketones decrease the O₂ cost of ATP synthesis, whilst certain amino acids protect mitochondria and cellular function in hypoxia (Figure 2.8). The advantages of the induced catabolism may also include an improved economy of movement following loss of body mass (Hopker et al., 2010), thinner myocytes resulting in enhanced capillary density and a possible improvement in oxygen delivery as a result of shorter diffusion distances. Furthermore a reduction in muscle mass results in loss of total mitochondrial mass further decreasing oxygen demand (Murray and Montgomery 2014).

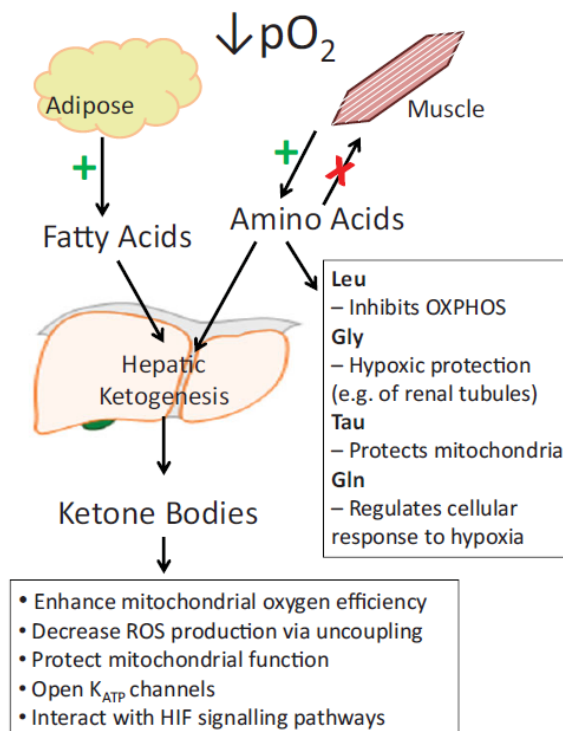


FIGURE 2.7: Fatty acids are released from adipose tissue by lipolysis whilst muscles break down releases amino acids. Fatty acids and some amino acids are converted into ketone bodies by the liver. Ketone bodies and amino acids act as metabolic substrates (Murray and Montgomery 2014).

Notes: Leucine (Leu), Glycine (Gly), Taurine (Tau), glutamine (Gln), Oxidative phosphorylation (OXPHOS), Reactive oxygen species (ROS), ATP-activated potassium channels (K_{ATP} channels).

Intermittent bouts of exposure rather than chronic exposures have also been observed to induce a lasting effect on metabolic factors (Quintero et al., 2010). Previously, intermittent exposures to hypoxia have reported, amongst a range of populations, benefits in terms of haematological status (Saunders et al., 2009) and cardiovascular functions including improved running economy, reduced lactate concentrations (Katayama et al., 2003) and improved cardiovascular performance (Hinckson et al. 2005; Hamlin and Hellems 2007). Quintero et al., (2010), following a study conducted on a population of rats, reported that a four week cycle hypoxia protocol consisting of four days hypoxia exposure separated by three days of normoxia triggered an appetite suppression and a continuous reduction in body weight gain for the subsequent three to five days with each new exposure. Perhaps most importantly the nature of the repeated intermittent bouts was seemingly able to eliminate any adaptation of the rats to the hypoxia as seen in chronic exposures and thus provided repeated alterations in appetite and body weight for a sustained period post-each exposure (Figure 2.9).

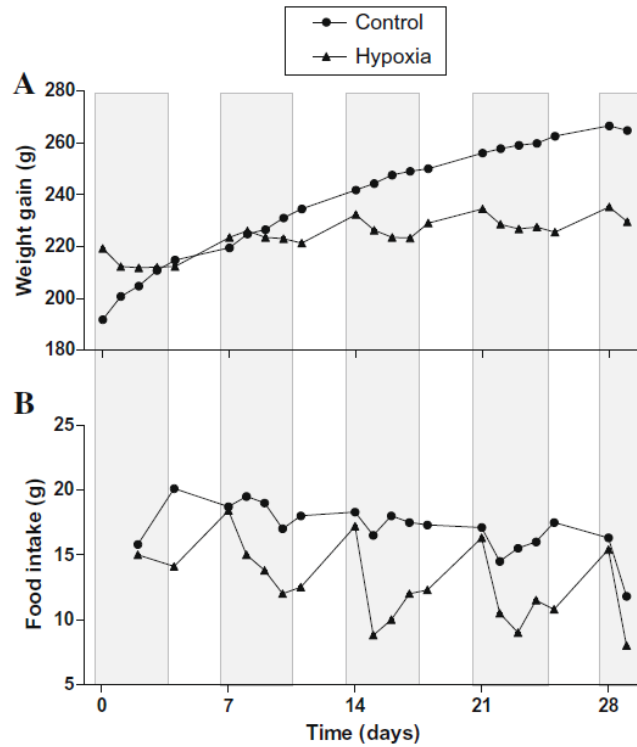


FIGURE 2.8: Weight gain (A) and food intake (B) over four weeks of cycle hypoxic (8% O₂) and normoxic exposure. Columns represent four days of hypoxia (Quintero et al., 2010).

Studies from Allahdadi et al., (2005), and Chen et al. (2005) both carried out on rodents have also reported weight loss following intermittent exposures to hypoxia further highlighting the potential in the method's use on human subjects. In contrast to more severe exposures the use of repeated intermittent exposures to hypoxic conditions supplies a more realistic and feasible opportunity, in terms of application, to gain the hypothesised benefits of exposure. Despite the lack of previous investigations into intermittent hypoxic exposure for weight loss amongst humans, the reported reduction in fat mass for animal modes, in which there are eight to date (Allahdadi et al., 2005; Chen et al., 2005; Dunleavy et al. 2005; Joyeux-Faure et al. 2005; Polotsky et al. 2003; Quintero et al., 2010 and Zoccal et al. 2008) supplies a sound rationale for the use of the method for human testing, albeit with severities and durations of hypoxic exposures feasible and ethical.

TABLE 2.2: Changes in body weight, body composition, and metabolic parameters in humans following exposure to altitude.

PROLONGED EXPOSURE				
AUTHOR	SAMPLE	DESIGN	HYPOXIA STIMULUS	EFFECTS
Consolazio et al. (1968)		Exposure to 4,300m for 28 days	Altitude	Total body mass loss of 2.66kg Losses of body fat N/A Losses of FFM N/A
Consolazio et al. (1972)		Exposure to 4,300m for six days	Altitude	Total body mass loss of 0.88kg Losses of body fat N/A Losses of FFM N/A
Hannon et al. (1976)	8	Exposure to 4,300m for seven days	Altitude	Total body mass loss of 1 kg Losses of body fat N/A Losses of FFM N/A

Boyer and Blume (1984)	14	Exposure to >5,400m for 23 days	Altitude	Total body mass loss of 4kg Losses of body fat 1.2kg Losses of FFM 2.8kg
Guilland and Klepping (1985)	4	Exposure to 4,800 – 6,000m for 20 days (trekking)	Altitude	Total body mass loss of 3.95 kg Losses of body fat N/A Losses of FFM N/A
Bradwell et al. (1986)	21	Exposure to 4,846m for 16 days	Altitude	Total body mass loss of 4.5kg Losses of body fat N/A Losses of FFM N/A
Worme et al. (1991)	8	Exposure to 2,400 – 4,300m for 31 days	Altitude	Total body mass loss of 1.9kg Losses of body fat 1kg Losses of FFM 0.9kg

Butterfield et al (1992)	7	Exposure to 4,300m for 21 days	Altitude	Total body mass loss of 2.2kg Losses of body fat N/A Losses of FFM N/A
Fulco et al (1992)	16	Exposure to 3,700 – 4,300m for 16 days	Altitude	Total body mass loss of 5.9 kg Losses of body fat 3.46 kg Losses of FFM 2.44kg
Westerterp et al. (1992)	5	Exposure to 5,300 – 8872m for 30 days	Altitude	Total body mass loss of 2.2kg Losses of body fat 1.4kg Losses of FFM 0.8kg
Westerterp et al. (1994)	6	Exposure to 6542m for 21 days	Altitude	Total body mass loss of 4.9kg Losses of body fat 3.5kg Losses of FFM 1.3kg

Pulfrey and Jones (1996)	6	Exposure to 5,900 – 8,046m for 40 days (trekking)	Altitude	Total body mass loss of 3.7 kg Losses of body fat 0.9 kg Losses of FFM 1.9kg
Armellini et al. (1997)	12	Exposure to 4,500m for 16 days (trekking)	Altitude	Total body mass loss of 3.3kg Losses of body fat 2.2kg Losses of FFM 1.1kg
Rose et al. (1988)	8	Progressive exposure up to 8846m for 38 days (trekking)	Simulated altitude (Hypobaric)	Total body mass loss of 7.4kg Losses of body fat 2.51kg Losses of FFM 5.05kg
Tanner and Stagger (1998)	5	Exposure to 2,200 – 4,300m for 21 days (trekking)	Altitude	Total body mass loss of 4.2kg Losses of body fat 3.2kg Losses of FFM 1kg

Reynolds et al.(1999)	10	Ascent of Everest – 8,848m over 63 days	Altitude	Body mass loss of 0.14kg.day ⁻¹ (8.82kg)
Westerterp-Plantenga et al. (1999)	8	Simulated ascent of Everest (8,000m) over 31 days . Measures at 0, 5,000, 6,000, 7,000 and 8,000m .	Simulated altitude (Hypobaric)	Total body mass loss at; 5,000m 2 kg 6,000m 2.8kg 7,000m 3.6kg 8,000m 4.7kg
Lippl et al. (2010)	20	Exposure to 2,650m for seven days	Altitude	Total body mass loss of 1.54 kg Losses of body fat N/A Losses of FFM N/A Increased BMR. Decreased HDL and DBP.
Macdonald et al. (2010)	41	Expedition lasting 21 days from SL to 5,100m	Altitude	Total body mass loss of 2.4 kg Losses of body fat 1.08 kg

				Losses of “residual mass” 0.48 kg
				Losses of TBW 0.84 kg
Wagner (2010)	8	Exposure up to 8,848m for 40 days (Simulated ascent of Everest)	Altitude	Total body mass loss of 9% BM Losses of body fat (33%) Losses of FFM (67% of total) Decreased CHOL, TAG
Wing-Gaia et al. (2014)	18	Exposure to 2,835 – 5,364m for 13 days (trekking)	Altitude	Total body mass loss of 1.9kg Losses of body fat 0.6kg Losses of FFM 1.2kg
TRAINING INTERVENTIONS				
AUTHOR	SAMPLE	DESIGN	HYPOXIA STIMULUS	EFFECTS
Bailey et al. (2000)	18 + 14 (H + C)	Cycling for 20-30 min/day, 3 x wk., 4 x week at intensity ranging from 70 – 85% HR max.	FiO₂: 16% . Simulated altitude (Normobaric)	Increased LBM (+ 1.4 ± 1.5kg) in H. Decreased max. SBP in H.

Haufe et al. (2008)	10 + 10 (H + C)	Treadmill running for 60 min/day, 3 x week., 4 weeks at HR corresponding to 3 mmol.L ⁻¹ .	FiO₂: 15% . Simulated altitude (Normobaric)	Greater decrease in body fat mass in H (3.5% reduction)
Netzer et al. (2008)	10 + 10 (H + S)	90 min/day, 3 x week., 8 weeks. Exercised (Stepper, Treadmill, Cycle ergometer) in either Sham or hypoxia at 60% HR max	FiO₂: 15% Simulated altitude (Normobaric)	Loss of Body Mass in H alone
Wiesner et al. (2009)	24 + 21 (H + C)	Treadmill running for 60 min/day, 3 x wk., 4 weeks at 65% $\dot{V}O_2$ max.	FiO₂: 15% Simulated altitude (Normobaric)	Decrease in body fat and increase in FFM greater in H compared to N. Numeric values N/A.

Notes: Studies have been separated by investigations in which either prolonged exposure to altitude/hypoxia occurred or in studies in which training intervention have been employed. Studies in which explicit information is provided regarding changes in body mass and body composition only are displayed. Only studies in which significant changes in body mass and/or body composition occurred have been included. Studies in which a deliberate energy deficit was employed in participants have been omitted from the Table. Studies are presented in chronological order. Hypoxia (H), control (C), sham (S), fraction of inspired oxygen (FiO₂), heart rate (HR), fat free mass (FFM), lean body mass (LBM), systolic blood pressure (SBP), diastolic blood pressure (DBP), high density lipoprotein (HDL), basal metabolic rate (BMR).

SUMMARY

Loss of body mass at altitude, particularly high altitude (> 5,000m) is common and in some cases considered impossible to avoid. In a review losses of between 5-15% at such altitudes have been outlined. Mean losses of body mass equating to approximately 150 – 200 g/day are reported with a contributory energy shortfall of 500kcal.day⁻¹ suggested as common, although greater deficits have been reported by a number of studies. There is some evidence to suggest that at high altitude lean tissue is the predominant contributor to losses of body mass whereas at more moderate altitudes loss of fat mass plays a greater role. Losses of body water through both increased urination and ventilation are also considered noteworthy in the short term.

At lower altitudes some studies have reported maintenance of energy balance and body mass through feeding and limited physical activity. However targeted increases in total energy and of diets with specific macronutrient contributions, including increased fat, CHO and protein intake, in humans at altitude have demonstrated varying results and levels of success. State of energy balance at altitude is integral to the observed changes with altered responses occurring in the adequately fed individual at altitude compared to the inadequately fed. Furthermore the severity of altitude induced cachexia may be sex-dependent with females demonstrating greater “adaptation” with relation to food consumption in comparison to males.

Other contributory factors to the extent of weight loss also include the altitude attained, the rate of ascent, and the duration of the stay along with level of physical activity and extremes in ambient temperatures. Nevertheless in studies attempting to isolate such confounding variables the independent effect of altitude/ hypoxia is considered causal in the observed alterations in humans exposed to an environment with reduced oxygen availability.

2.8 HYPOXIA AND FAT METABOLISM

OVERVIEW OF FAT METABOLISM

Triglycerides consist of three fatty acids chemically combined with a glycerol molecule. Excess carbohydrates, proteins and fats are converted and stored as TAG in the body which are broken down by the process of lipolysis into its forming components (fatty acids and glycerol). The process of fatty acid catabolism occurs in the matrix of the mitochondria which provides a greater yield of ATP in comparison to the breakdown of glycerol. Following beta oxidation of fatty acids Acetyl coenzyme A (acetyl-CoA) transports the fuel molecules being oxidised to the Krebs cycle for the generation of ATP.

Fat and carbohydrate are the dominant substrates for ATP production in skeletal muscle during aerobic exercise in well fed humans (Spriet, 2002). With the potential to represent an unlimited source of fuel for oxidation by skeletal muscle the largest nutrient chemical store of energy used to power biological processes are lipids (Hawley et al., 2001). Triglyceride is the principal store of lipids in

adipose tissue along with a further important store found within skeletal muscle adjacent to the mitochondria referred to as intramuscular triglycerides (IMTG). Finally dietary-derived fatty acids during exercise can be derived from plasma triacylglycerol (chylomicrons) and very low-density lipoproteins (VLDL).

The majority of FFAs are transported across the muscle membrane by transport proteins with a smaller portion travelling across the membrane by diffusion. Binding with coenzyme A activates FFA following transport to the surface of the outer mitochondria membrane from the cytoplasm before being converted to a fatty acyl carnitine compound. This compound is then transported across the mitochondrial membranes whilst bound to carnitine before the carnitine is removed inside the mitochondria. Along with the removal of the carnitine the coenzyme A is rebound and the fatty acyl-CoA molecules are metabolised in the beta oxidation pathway (Spriet, 2002). Beta oxidation involves the removal of sequential pairs of carbon atoms of the fatty acids by dehydration, hydration and cleavage reactions with the resulting two carbon fragment then being attached to coenzyme A forming the aforementioned Acetyl Coenzyme A. This metabolism occurs with the production of reducing equivalents NADH, FADH₂ and acetyl-CoA. The acetyl-CoA is further metabolised in the tricarboxylic (TCA) pathway producing additional reduction equivalents which are then accepted by the electron transport chain (including oxygen) to generate the proton motive force which provides the chemical energy used to synthesise ATP from inorganic phosphate (Pi) and ADP in the process of oxidative phosphorylation (Hawley et al., 2001).

Interestingly aerobic training increases the absolute rate of energy production from the oxidation of fat in skeletal muscle resulting in an increased power output and/or time that aerobic exercise can be maintained before the consumption of carbohydrate stores. Of importance for maintaining the delivery of FFA to the working muscle during exercise is the ability of adipose tissue to respond by activating TAG lipolysis and release FFA into the blood. During exercise, increases in plasma noradrenaline and adrenaline override the inhibition of lipolysis by the same hormones when at lower concentrations. This results in an activation of the hormone sensitive lipase (HSL) and TAG breakdown to FFA and glycerol from which point the FFA must then be released from the adipose tissue to the blood. Delivery of FFA to the working muscle is a function of the plasma and muscle blood flow which increases with increased power during exercise thus resulting in a several fold increase in FFA delivery to the muscle. During prolonged, low-to moderate-intensity exercise blood FFA increases contributing to increased FFA delivery. Although the relationship between muscle FFA delivery and plasma FFA uptake is not linear it has been found to influence FFA oxidation.

2.9 EFFECT OF HYPOXIA ON LIPOLYSIS

Short term exposure to sustained hypoxia has induced hypertriglyceridemia in humans (Barnholt et al., 2006; Farias et al., 2006). Hypertriglyceridemia results from increased supply and impaired removal of triglyceride rich lipoproteins from circulation (van Hoek et al., 2009). It has been

hypothesised that these elevations in TAG can be explained by similar mechanisms in which chronic intermittent exposures to hypoxia have been shown to increase TAG levels in mice; namely an increased hepatic secretion and decreased lipoprotein clearance (Jun et al., 2012). Increased sympathetic activity upon exposure to hypoxia may also play a role through the activation of hormone sensitive lipase and thus drive the release of fat. The rise in TAG and FFA should be expected if the removal by oxidation and uptake does not increase to the same extent.

In a study from Jun et al. (2012) chronic intermittent exposure to graded hypoxic exposures (FiO_2 : 0.21, 0.17, 0.14, 0.10, 0.07) induced increases in both plasma and liver TAG levels and were correlated with increasing severities of FiO_2 . Interestingly the same study reported that an exposure of FiO_2 : 0.12 induced white adipose tissue lipolysis in mice whereas more severe hypoxia (FiO_2 : 0.07) was not seen to consistently stimulate lipolysis.

In an attempt to account for the effects of TAG changes over time, levels of TAG were assessed at 2 hour intervals during exposure to both FiO_2 : 0.21 and 0.10. As expected, TAG levels reduced as the length of time in a fasted state increased. Crucially however this was seen to occur more slowly in mice exposed to hypoxia and thus it was concluded that exposure to acute hypoxia rapidly delays postprandial TAG clearance with this effect then normalised as oxygen levels are restored (Jun et al., 2012). This effect was attributed to a reduction in brown and white adipose tissue lipoprotein lipase activity and was seen in conjunction with a decreased uptake and oxidation of FFA. Also of interest the study, contrary to its initial hypothesis found an inhibition in the hepatic secretion of TAG following acute hypoxic exposure.

The primary purpose of brown adipose tissue is to produce heat through the use of FFA and TAG which is highlighted by the increased clearance of TAG in plasma in cold conditions which is reviewed more thoroughly elsewhere in this review of literature. When exposed to hypoxic conditions small mammals such as mice enter a state of regulated hypothermia in order to reduce their consumption of oxygen which is primarily achieved through the inactivation of brown adipose tissue (Jun et al., 2012). Therefore it is likely that hypoxic exposure suppressed lipoprotein lipase activity and the transport of FFA and that inactivation of brown adipose tissue are accompanied by hypertriglyceridemia and decreased lipid uptake via lipoprotein lipase.

Previously discussed in this review of literature is the effect of energy balance on changes in body mass in humans exposed to altitude. Similarly, state of energy balance has also been seen to be influential on fat metabolism under conditions of hypoxia. In a complex study in which the effects of calorie restriction and chronic hypoxic exposure were investigated in comparison to energy deficient individuals exposed to the same environment (4,300m for 21 days) (Barnholt et al., 2006), several important differences between the two groups were highlighted. Both glucose and insulin were transiently increased at altitude in both deficiently and adequately fed participants, a finding previously reported with increased elevation. This reported rise was less so in the energy deficient

group however suggesting a restriction of calorie intake at altitude dampens the usual rise in these two measures with increased elevation. This effect is in light in previous studies in which calorie restriction in isolation of altitude has been shown to reduce glucose and insulin concentrations. Lower blood glucose concentrations contribute to a shift in substrate utilisation from carbohydrate to alternative fuel sources being used namely ketones and fats. Interestingly when individuals are exposed to altitude coupled with an energy deficit a greater reliance on lipid metabolism has been recorded (Young et al., 1989) in an attempt to spare the limited carbohydrate stores. However when weight is maintained stable through sufficient diet a lower use of lipid stores is most commonly seen (Barnholt et al., 2006).

In the face of disparate results regarding the influence of altitude exposure on substrate selection (Kennedy et al., 2001) examined the effects of both acute (24hr) and chronic (five weeks) altitude exposure on key enzymes involved in fat metabolism. It is generally accepted that fatty acid oxidation is largely regulated by the activity of CPT-I and the transport of long chain fatty acyl groups into the mitochondria. Kennedy et al., (2001) reported significant reductions in the activity of CPT-I in the heart and in the extensor digitorum longus muscle for both acute and chronic exposures to altitude in rats compared with controls. This down-regulation of CPT-I suggests a decreased capacity for fatty acid oxidation and suggests a subsequent increase in CHO dependency as a fuel. Kennedy also observed reduced β -HAD (a marker of β -oxidation) activity in both the heart and liver following the chronic exposure to simulated altitude of 4,300m consistent with the concept of greater reliance on CHO at altitude. In a study from Katayama et al. (2009) in which exercise at a simulated moderate altitude of 2,000m was conducted a reduction in glycerol and FFA were observed during recovery at altitude compared to sea-level. Such findings may suggest a reduction in lipolysis at altitude, in this case during recovery from exercise. Mechanistic explanations for such findings include an increase in insulin concentration which has been shown to diminish adipose tissue lipolysis (Lafontan and Berlan 1993) similarly an increase in lactate concentration has also been shown to diminish lipolysis and FFA mobilisation (Boyd et al., 1974). Thirdly a possible increase in growth hormone factor induced by hypoxia and exercise may also alter lipolysis at altitude (Lafontan and Berlan 1993).

2.10 INSULIN RESISTANCE AND SENSITIVITY AT ALTITUDE

OVERVIEW

Insulin circulates at levels proportional to fat mass and can cross the blood brain barrier. Insulin within the brain acts as an anorexigenic signal but levels are also sensitive to the acute effects of food ingestion and its principal function is control of glucose homeostasis rather than the control of body mass (Woods and Porte Jr. 1983). Increased fasting plasma glucose concentrations in the presence of increased plasma insulin concentrations indicate insulin resistance. The insulin resistance may be located in the liver or in the peripheral tissues; such as in the skeletal muscle and adipose tissue, or in a combination of both (Larsen et al., 1997). Insulin resistance in the liver would result in an elevation

of the hepatic glucose output whereas resistance in the peripheral tissues would lead to a decrease in glucose clearance. In order to compensate for resistance regardless of its cause, insulin secretion would increase in order to compensate for the resistance thus increases insulin concentration in the blood will occur (Larsen et al., 1997).

Although it is frequently reported that diseases characterised by chronic hypoxic episodes are associated with glucose intolerance (Brooks et al., 1994) the current literature does not draw conclusive associations (Oltmanns et al., 2004). Within the available literature there appears to be differences between the effect of acute and long term exposure to hypoxic conditions with the effect of glucose intolerance seeming to disappear with a more prolonged effect of hypoxia (Brooks et al., 1991). Hypobaric hypoxia, as previously discussed, has been shown to be associated with increased sympathetic activity which in turn may have a substantial effect on glucose and lipid metabolism (Kelly et al., 2010). Acute exposure to $\geq 3,000\text{m}$, in contrast to more prolonged stays, has, in some studies, resulted in a reduction in insulin sensitivity (Louis and Punjabi 2009; Braun 2008; Oltmanns et al. 2004). Furthermore the majority of previous literature suggests that adverse blood glucose regulation was noted in studies with either acute exposure to high altitude ($> 4,000\text{m}$) or high intensity training (Mawson et al. 2000; Oltmanns et al. 2004; Louis and Punjabi 2009). This however is not conclusive as glucose metabolism has been seen to be enhanced as shown on two studies that reported a 36-60% improvement in glucose uptake (Kelly et al., 2010; Wiesner et al., 2010). Alternatively there is also evidence to suggest that endurance training completed in hypoxia has a beneficial effect on insulin sensitivity in comparison to matched exercise in normoxic conditions. The following section will highlight the effect of short and longer term effects of exposure to hypoxia on insulin and examine the use of exercise coupled with exposure to hypoxia on the same parameters.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

In a study from Kelly et al. (2010) the effects of acute altitude induced hypoxia on glucose metabolism and plasma hormone responses after glucose ingestion was examined in healthy participants. It was hypothesised that blood glucose would be suppressed at altitude because of the combined effect of insulin and hypoxia on peripheral glucose uptake in healthy insulin sensitive individuals. The study provided novelty in that the acute metabolic responses to hypoxia were observed independent of acclimation, obesity, insulin resistance and/or weight loss in healthy adults. It is known that insulin and hypoxia stimulate glucose transporter (GLUT) 4-mediated glucose transport via separate signalling pathways (Cartee et al., 1991). Hypoxia induces glucose uptake via a calcium-dependent and insulin-independent pathway that involves the stimulation of GLUT4 translocation to the plasma membrane with an accompanied increase in glucose uptake (Cartee et al., 1991). In contrast insulin-mediated glucose transport is achieved through the stimulation of a separate pool of GLUT4 glucose transporters (Douen et al., 1990). The study found that a single simulated altitude exposure of 4,300m resulted in a lower glucose response after ingestion of 75g of glucose compared to the ingestion of

the same amount of glucose in normoxic conditions. Interestingly the glucose response was observed without an alteration in the insulin response or insulin secretion which was measured by changes in C-peptide levels. In contrast; work from Oltmanns et al. (2004) found that a 30 minute exposure to hypoxia (oxygen saturation decreased to 75%) impaired insulin action during a euglycemic clamp. It is suggested that likely causes for the difference in findings may be the timings and durations of the hypoxic exposure. Furthermore the ingestion of glucose compared with the infusion of glucose during a clamp provides different physiological stimuli (Kelly et al., 2010). Future research may focus on euglycemic clamp studies whilst in an altitude chamber in order to provide a more definitive answer regarding the effects of acute hypoxic exposure on peripheral and hepatic insulin sensitivity. It is likely that the decrease in plasma glucose during the altitude trial is due to an increase in glucose uptake and metabolism by the muscle rather than due to the observed increase in adrenaline even though increases in adrenaline can inhibit peripheral insulin action (Lager et al., 1986).

Oltmanns et al. (2004) demonstrated that hypoxia acutely induces glucose intolerance providing evidence for a causative role of hypoxia. The study in which hypoglycaemic clamp experiments in fourteen healthy males were conducted in hypoxic and normoxic conditions separated by four weeks, also reported the decreased glucose infusion rate during hypoxia in conjunction with an increase in adrenaline concentrations and symptoms of anxiety. These results however were unable to determine whether the glucose intolerance was secondary to hypoxia or to the increases in adrenaline and anxiety. As hypoglycaemia is known to induce glucose intolerance as well as increases in catecholamine and cortisol concentrations (Attvall et al., 1987) the study had rationalised that if glucose intolerance occurred solely due to the rise in stress hormones on exposure to hypoxia; then stronger effects on glucose metabolism after hypoglycaemia would be expected than after hypoxia. Although clear differences in stress hormones were observed, glucose intolerance was closely comparable between hypoxic and hypoglycaemic conditions (Oltmanns et al., 2004).

It has been suggested that the altitude induced stimulation of adrenaline has the possibility to elicit insulin resistance. Furthermore impaired oxidative capacity and muscular mitochondrial insufficiency may also contribute (Wee and Climestein 2013). An apparent insulin resistance was reported by Larsen et al. (1997) following two days at an altitude of 4,559m during a glucose clamp in healthy men, and in women by Braun et al., (2001) 10 days after arrival on Pike's Peak (4,300 m) during a food tolerance test. The study from Larsen clearly showed that insulin action is markedly reduced after two days at altitude and that following an additional five days at altitude insulin action is partly restored. These results are not directly comparable to those from the aforementioned Kelly et al. (2010) study however due to the physiological adaptations that may have occurred during the two days acclimatisation whilst at altitude (Kelly et al., 2010). The study also observed similar rates of glucose appearance at sea level and at 4,559m thus concluding that hepatic glucose production is not influenced by altitude (Larsen et al., 1997). The decreased insulin action was partly explained by the

increase in plasma cortisol concentrations as cortisol administration has been seen to induce peripheral insulin resistance due to a decrease in glycogen synthase enzyme activity in skeletal muscle (Holmäng and Björntorp 1992). In accordance with this, improvements in insulin action were observed at day 7 in the Larsen study, when plasma cortisol concentration had returned to sea level values. Furthermore, plasma adrenaline concentrations increased more in response to insulin infusion following two days at altitude compared to sea-level values. The authors postulated (Larsen et al., 1997) that adrenaline inhibits peripheral insulin action via β_2 –receptors (Lager et al., 1986) and thus may have contributed to the observed decrease in peripheral insulin action following two days exposure although the contribution is likely to be of minor importance. It was concluded therefore that increases in concentrations of glucose and insulin during altitude occur as a consequence of a transient peripheral insulin resistance (Larsen et al., 1997).

In a study aiming to investigate the effects of acute exposure to moderate altitude on vascular function, metabolism and systemic inflammation, 51 healthy males were exposed to 2,590m for two days. Metabolic markers including insulin resistance from the HOMA-index were measured (Stöwhas et al., 2013). The homeostatic model assessment or the HOMA-index is a widely used method for the estimation of insulin resistance and beta-cell function in research developed by (Matthews et al., 1985). It is calculated multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG), then dividing by the constant 22.5, i.e. $HOMA-IR = (FPI \times FPG) / 22.5$. No significant effect on glucose metabolism or insulin resistance was observed following two days at 2,590m. The effects of hiking activities on glucose tolerance has also been examined (Lee et al., 2003). Nine untrained participants were tested before and after a three day stay at an altitude of 2,400m, and in contrast to most acute investigations observed an improved glucose tolerance (Lee et al., 2003). The independent effect of exercise on glucose transportation however must be considered in studies in which physical activity is undertaken. During exercise glucose is transported into skeletal muscle to a substantial extent independent of insulin (Oltmanns et al., 2004). The effect of exercise may therefore confound any estimate of glucose intolerance under conditions of hypoxia. It is also important to consider in studies highlighting improvements from short term training interventions that an acute effect of the final training session may result in improvements in insulin sensitivity that may not be seen were the measure taken 48 hours post the final session (Whyte et al., 2010). This is due to the acute effect of exercise on insulin sensitivity. Work from Debevec et al., (2014) examined the effect of a 10 day hypoxic confinement coupled with exercise versus the effect of hypoxia alone on various metabolic markers. It was observed that following hypoxic confinement insulin area under the curve (AUC) was reduced in both groups following the ten day study period suggesting that hypoxia per se beneficially affects postprandial insulin (Debevec et al., 2014).

In a study from Wiesner et al. (2010) fasting insulin decreased after training in both normoxic and hypoxic groups suggesting hypoxia did not have a major exercise independent effect on fasting insulin and glucose metabolism in obese participants. A recent investigation examining the effects of differing

periods of hypoxic training on glucose metabolism and insulin sensitivity reported that longer term interventions compared to shorter duration interventions with equivalent training may be more beneficial for improving insulin sensitivity (Morishima et al., 2015). Specifically the aim of the investigation was to examine the effects of normobaric hypoxic training (FiO₂: 0.15) lasting for either two weeks, during which sedentary males cycled at 65% $\dot{V}O_2$ max six times a week, or four weeks, during which exercise sessions were completed three times a week. Results indicate that AUC serum insulin response to glucose ingestion during an oral glucose tolerance test was reduced by hypoxic training for the four week intervention but not the two week intervention. Reductions from 6910 ± 763 to 5812 ± 663 $\mu\text{IU ml}^{-1}$ demonstrate the findings. It is suggested that these outcomes are a combined effect of the endurance training and the hypoxic exposure. Hypoxic exposure has been seen to improve insulin sensitivity in diabetic individuals (Mackenzie et al., 2012). Endurance training improves insulin sensitivity mediated by changes in components of the insulin signalling pathways that result in greater insulin induced translocation of GLUT-4 to the cell surface (Goodyear et al., 1992).

It has been suggested that greater glucose disposal rates combined with unchanged insulin levels imply increased insulin sensitivity following longer stays at altitude (Mazzeo et al., 1991). It has also been reported that chronic hypoxia increases dependence on glucose in men and increases insulin sensitivity in men and women (Marquez et al., 2013). This is further supported from Schobersberger et al. (2003) in a study in which 22 male participants with metabolic syndrome were exposed for three weeks to a moderate altitude of 1,700m during which glycaemic parameters and measures of lipid metabolism were monitored during a simulated holiday with moderate sports activities. Metabolic syndrome is classified as a disease where patients exhibit a combination of obesity, hypertension, dyslipoproteinemia and glucose intolerance (Schobersberger et al., 2003). The syndrome is strongly related to an increase in sympathetic nervous activity leading to an increased cardiovascular risk (Schobersberger et al., 2003). It was rationalised in the study that acclimatisation to conditions of moderate hypoxia, combined with moderate physical exercise, may improve symptoms of the metabolic syndrome by causing reduction in sympathetic tone. The main aim of the study was to investigate the metabolic changes focusing on insulin resistance using the HOMA index before, during and one and six weeks after the prolonged stay in the Austrian Alps. Significant reductions in the HOMA index were observed and used as a measure of improved insulin resistance upon return to 500m following the three weeks of altitude stay (Schobersberger et al., 2003). The effect of the moderate physical activity undertaken during the sojourn, which included walking tours four to five times a week, cannot be excluded as a possible reason for the changes in HOMA index as endurance exercise has been seen to acutely increase HDL cholesterol and possibly improve insulin resistance (Thompson et al., 2001). The exact mechanisms underlying the possible influence of moderate hypoxia on these changes however are still relatively unclear. As previously outlined it has been suggested that hypoxia induced changes in key enzymes in fat utilisation and oxidation are involved (Kennedy et

al., 2001) and that the marked reduced activity of insulin seen upon acute exposure to altitude may partly be explained by changes in counter-regulatory hormones (Larsen et al., 1997).

Chronic exposure to high altitude (> 3 weeks) coupled with moderate levels of physical activity has showed favourable blood glucose responses in unhealthy participants (de Mol et al., 2012; Wiesner et al., 2010). These findings suggest that positive adaptations in blood glucose regulation require extended exposures at moderate levels of altitude. Furthermore differences in the effects of blood glucose may be attributed to the varying levels of hypoxia used in combination with differing exercise intensities. In an investigation from Marquez et al. (2013) the hypothesis that markers of glucose metabolism would change with cyclic variations in altitude conditioning was tested. Two groups, both consisting of middle aged men (48 ± 6 years) considered to be at risk for metabolic syndrome, were exposed to 10 weeks for 40 minutes a day, three times per week to either cyclic hypobaric hypoxia or sham. Individuals in the hypoxic group were exposed to cyclic pressures simulating altitudes ranging from sea level to 3,048m in week one progressing to 6,096m by week five and continued at this range until week 10. Measures of physical function and blood markers of glucose metabolism were measured at baseline and at week three, six and 10. Body mass remained stable throughout the 10 week investigation. A reduction in plasma glucose response to an oral glucose tolerance test was greater in the cyclic hypoxic group compared to sham following 10 weeks. Neither group experienced changes in fasting insulin, insulin response during the oral glucose tolerance test or changes in a timed walk test. It was concluded from the study that cyclic hypobaric hypoxia improves markers of glucose metabolism in middle-aged men at risk of metabolic syndrome. Mechanisms behind these changes remain unclear however it is suggested that hypoxia induced changes in key enzymes in fat utilisation and oxidation may be attributing factors (Schobersberger et al., 2003).

Intermittent exercise can encourage blood glucose removal and previous studies have also reported that training under hypoxic conditions is capable of improving metabolic status and glycaemic control in healthy individuals (Haufe et al., 2008; Morishima et al., 2014a) and clinical populations (Mackenzie et al., 2012). It is reasonable to speculate from these studies that intermittent exercise in hypoxia has an additive effect in comparison to intermittent exercise alone as the recovery passive phase in hypoxia may further stimulate glucose uptake. Given the increased glucose use during exercise at altitude it remains a source of debate with regards to what an apparent resistance to insulin actually means.

SUMMARY

Exposures to hypoxia of both short and longer term have been seen to induce alterations in insulin concentration and sensitivity. The existing research however is conflicting and results are confounded by differences in the length and extremity of exposure and the inclusion of physical activity.

It is suggested that hypoxia acutely induces glucose intolerance and that the altitude induced stimulation of adrenaline may impair insulin action and thus elicit insulin resistance. Long term exposure to moderate hypoxia has however been suggested to increase insulin sensitivity although the exact mechanisms are unclear. There is also growing evidence to suggest that hypoxia coupled with physical activity has a synergistic beneficial effect on insulin sensitivity. Future work is required however in order to further examine the effects of hypoxia coupled with physical activity of various modes and intensities. The lasting effect of prolonged exposure to altitude is also a worthy cause of investigation.

2.11 BROWN ADIPOSE TISSUE AND METEORIN –LIKE PROTEIN (METRNL)

OVERVIEW

Brown adipose tissue (BAT) is associated with the maintenance of core temperature in humans. The most important feature of brown fat is its capability to oxidise substrates to produce heat (Cannon and Nedergaard, 2004). These thermogenic attributes has led to the suggestion that BAT and the recently discovered “beige” adipose tissue may be useful in combating obesity and its related metabolic disorders (Van Marken Lichtenbelt et al., 2009).

Exposure to cold conditions, and regular physical activity, expands the thermogenic capacity of BAT by increasing the amount of brown adipocytes in the tissue (Hao et al., 2014) and by recruiting “beige” cells in white adipose tissue (Cannon and Nedergaard 2004). This “browning” of certain populations of white adipocytes may result in a more brown-like adipocyte that is capable of thermogenesis through increased expression of uncoupling protein 1 (Warner and Mittag 2015). An observed uptake and combustion of glucose and lipid within the tissue (Bartelt et al., 2011; Van Marken Lichtenbelt et al., 2009), improved insulin sensitivity (Stanford et al., 2013) and increased EE (Lowell and Spiegelman 2000) are reported highlighting BAT as a possible mediator in the treatment and prevention of metabolic disorders (Cannon and Nedergaard 2004). It has also been suggested that BAT most likely played an important role in the evolutionary success of mammals due its enhancement of an active life in cold surroundings. The underlying mechanisms for the triggers of fat “browning” are however poorly understood. It also currently remains unclear whether “beige” adipocytes can sufficiently contribute to whole-body EE in a manner that is functionally significant to humans (Warner and Mittag 2015).

PGC-1 α is a transcriptional coactivator induced by exercise that controls the genes involved in oxidative metabolism and mitochondrial biogenesis, a novel form of which (PGC-1 α 4) has been identified and seen to be highly expressed in skeletal muscle particularly during exercise in mice and humans (Ruas et al., 2012). The expression of PGC-1 α 4 in skeletal muscle stimulates increased mRNA expression and secretion of the hormone Meteorin-like (Metrnl) which has been observed to lead to the “browning” of white fat (Rao et al., 2014).

Observed increases in *Metnl* expression are reported following exposure to acute cold conditions (Rao et al., 2014). Such findings highlight the potential for extremes in ambient temperature and other environmental conditions to promote its expression, and subsequent fat browning effects. Increased expression of *Metnl* through exposure to the cold and other environmental extremes such as altitude may therefore provide a method of fat browning and increased thermogenesis in humans. The aforementioned findings from Rao et al. (2014) and work investigating the combined effects of both cold and altitude on the thermogenic response highlight the importance of ambient temperature to these hypothesised changes in BAT activity (Blatteis & Lutherer 1976; Gautier et al. 1991; Cadena & Tattersall 2014).

The following sections will initially outline the functional significance and physiological differences between the three main types of adipose tissue in the human body. An overview of the recent research that has centred on BAT with regards to preventing and treating obesity and its associated disorders will then be presented. Furthermore triggers and possible mechanistic explanations regarding the “browning” of adipose tissue will be explored including exposure to extreme environmental conditions and physical activity. Most notably the effect of Meteorin-like protein will be reviewed as a mechanism for fat browning during exposure to cold conditions.

2.11.1 TYPES OF ADIPOCYTES

Adipose tissue functions not only as an energy storage depot but also as an endocrine organ secreting numerous adipokines acting on the brain, liver, muscle and other tissues (Cannon and Nedergaard, 2004; Kershaw and Flier, 2004). Adipose tissue can be classified into white adipose tissue (WAT) and brown adipose tissue (BAT) each of which having a different role in humans. A third type of fat has also recently been identified in rodents (Wu et al., 2013) termed beige or brite (brown in white) fat. Beige fat cells are interspersed in the WAT of humans and rodents (Wu et al., 2013; Stanford et al., 2015).

WAT represents the main energy storage in the body during feeding and the release of lipids during starvation. WAT is also generally considered as insulation against the cold and as a safe storage site of fatty acids that have been esterified into triglycerides. WAT is characterised by large white adipocytes which contain few mitochondria and is located mainly in subcutaneous (under the skin) and visceral (associated with the digestive tract) locations in humans (Stanford et al., 2015) WAT may also be found however around internal organs including the heart, kidneys, lungs and arteries. Once the storage capacity of WAT is exceeded the clearance of fatty acids from the systemic circulation is prevented resulting in an accumulation of these fatty acids in other organs including the liver and muscle which can subsequently lead to insulin resistance and thus type 2 diabetes and/ or cardiovascular disease (Speakman and O’Rahilly 2012).

Brown adipose tissue is specialised in EE and is dedicated to the generation of heat via the burning of lipid. It is characterised by smaller lipid droplets and a high number of mitochondria when compared to WAT. Brown adipose tissue itself is a relatively recent discovery in human physiology (Warner and Mittag 2015). Within the last century it was determined that BAT is found in all mammals and even more recently it was understood that heat production is one of its functions. Specifically brown adipose tissue is integral in the process of both non-shivering thermogenesis and for the cold-acclimation recruited noradrenaline-induced thermogenesis (Cannon and Nedergaard 2004). As such BAT is activated when additional heat production is required for the maintenance of core body temperature (Dijk et al., 2015) with its main function being to transfer energy from food into heat (Cannon and Nedergaard 2004). Although obesity is characterised by an expansion of adipose tissue mass, BAT is inversely correlated with BMI in humans (Cypess et al., 2009) in part due to its consumption of large amounts of energy for thermogenesis (Lowell and Spiegelman 2000). Brown adipose tissue is a highly energetic organ that utilises glucose and fatty acids as fuel (Bartelt et al., 2011; Van Marken Lichtenbelt et al., 2009) and in adult humans substantial depots of metabolically active BAT are present. This has led to the suggestion that BAT may play an important role in the maintenance of metabolic health and the lean phenotype (Stanford et al., 2013).

2.12 BROWN ADIPOSE TISSUE AND THERMOGENESIS

Upon exposure to cold conditions changes in the partitioning of energy substrates occur. Namely BAT is activated, and plays a major role in non-shivering thermogenesis (Hao et al., 2014). In order to fuel non-shivering thermogenesis fatty acids are “re-routed” to brown adipose tissue as the functional thermogenic unit of brown adipose tissue is the brown adipocyte itself (Cannon and Nedergaard 2004). Cold exposure or beta-adrenergic activation of BAT causes lipolysis in brown adipocytes thus during thermogenesis brown adipocytes take up large amounts of lipids and glucose from the circulation (Bartelt et al., 2011) as there is an increased supply. As such fatty acids are a major contributor to BAT thermogenesis via beta-oxidation (Yu et al., 2002). An activation of brown adipose tissue is also observed following diets consisting of low protein through a Leptin-dependent recruitment of the tissue (Warner and Mittag 2015).

Although proposed explanations exist the underpinning mechanisms for the energy redistribution of substrates to BAT remain largely unknown (Dijk et al., 2015). More established is the acute control of BAT activity. The rate of thermogenesis is controlled centrally via a pathway initiated in the hypothalamus – (Cannon and Nedergaard 2004). An area of the brain most likely to be the ventromedial hypothalamic nucleus acutely controls BAT activity. This part of the brain coordinates information regarding body temperature, feeding status and body energy reserves. Signals via the sympathetic nervous system are sent to the individual brown adipocytes during times when there is reason to increase the rate of heat production or increase the rate of food combustion. Noradrenaline is released and initiates the breakdown of triglycerides in brown adipocytes primarily via beta 3

adrenergic receptors. Fatty acids are thus released from triglycerides, both of which are considered acute substrates for thermogenesis. The release of free fatty acids derived from circulating triglyceride rich lipoproteins is catalysed by the enzyme lipoprotein lipase (LPL), which is highly abundant in BAT (Bartelt et al., 2011; Dijk et al., 2015) cold exposure stimulates LPL activity in BAT thus causing an increase in triglyceride rich lipoproteins fatty acid uptake and uptake of whole lipoprotein particles. The specific mechanisms for the increased LPL activity in BAT upon cold exposure however remain incompletely defined (Carneheim et al., 1988). Triglycerides and fatty acids are also, in part, regulators of the activity of uncoupling protein-1 (UCP1). An uncoupling protein is a protein found in the inner membrane of the mitochondria which aids in providing energy for oxidative phosphorylation (a metabolic pathway in which ATP is reformed). UCP1 specifically is highly abundant in BAT and mediates the uncoupling process (Cannon and Nedergaard 2004; Dijk et al., 2015)

The mitochondrial protein uncoupling protein-1 (UCP1, thermogenin) is responsible for the function of brown adipose tissue (Cannon and Nedergaard 2004) and is essential in the process of thermogenesis in brown adipocytes and for non-shivering thermogenesis in animals. Increased heat production occurs through an increased fraction of the food and oxygen available being taken up by the tissue and combusted within the tissue. UCP1 allows for the mitochondrial combustion of substrates uncoupled from the production of ATP following combustion of fatty acids in the respiratory chain/ electron transport chain. An overview of the acute control of brown adipose tissue activity is highlighted in Figure 2.10.

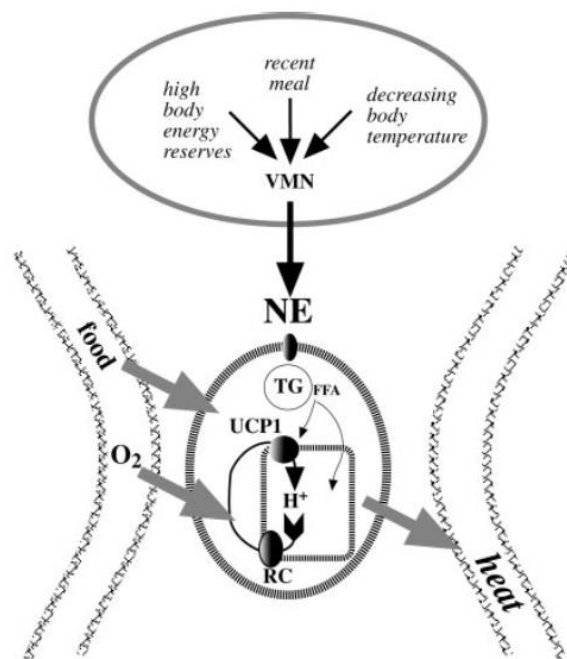


FIGURE 2.9: An overview of the acute control of brown adipose tissue activity (Cannon, 2004).

Notes: Ventromedial hypothalamic nucleus (VMN), Noradrenaline (NE), Triglycerides (TG), free fatty acids (FFA), Uncoupling protein-1 (UCP1), Hydrogen (H⁺) respiratory chain (RC), Oxygen (O₂).

Although incompletely defined a suggested mechanism for the redistribution of energy substrates to BAT is that of Angiopoietin-like 4 (ANGPTL4). Angiopoietin-like 4 is a protein that inhibits lipoprotein lipase activity and is highly expressed in BAT. It has been demonstrated that ANGPTL4 is part of a shuttling mechanism that directs fatty acids derived from circulating triglyceride-rich lipoproteins to BAT during exposure to the cold (Dijk et al., 2015). Specifically during sustained cold it was shown that in white adipose tissue an up-regulation of ANGPTL4 occurs preventing a cold-induced increase in LPL activity. Conversely in BAT a down regulation of ANGPTL4 is observed likely due to AMPK activation and thus an enhancement of LPL activity and uptake of plasma triglyceride derived fatty acids. These findings suggest an important role of ANGPTL4 in regulating plasma lipid partitioning during sustained cold.

As previously mentioned in this review of literature a shift toward non-shivering thermogenesis is observed in mammals as a physiological adjustment during cold acclimation (Hart et al., 1956) primarily in response to an upregulation of UCP1 and an increase in brown adipose tissue size. As a result an increase in BMR is observed with acclimation to cold (Benzinger, 1969; Claessens-van Ooijen et al., 2006; Marriott and Carlson, 1996). In addition to cold exposure alone, the combination of extreme environmental stimuli on the thermogenic response, namely hypoxia and cold exposure, has also received some attention (Blatteis & Lutherer 1976; Gautier et al. 1991; Cadena & Tattersall 2014). In summary work in both man and rodents has identified an altered thermogenic response to a combination of the two environment stimuli compared to cold exposure alone. Specifically an altitude induced blunting of the non-shivering thermogenic response to cold conditions.

Specifically, findings demonstrate that in both lowlanders and high altitude natives, altitude exposure to 3,350 and 4,340m reduced the calorogenic response to cold (three hours at 10°C) measured through VO₂ with this effect seemingly unaltered by acclimatisation to altitude, yet reversible immediately on descent to sea-level (Blatteis & Lutherer 1976). Similarly, work from Gautier et al. (1991), speculated that hypoxia inhibits the thermogenic response to cold conditions in both cold-acclimated and non-acclimated rats. This speculation was based upon a transient decrease in shivering and a sustained decrease in non-shivering thermogenesis associated with a decreased body temperature when exposed to an environment of FiO₂:0.12 and 5°C.

The effect of hypoxia on BAT thermogenesis has more recently been investigated in a rat model (Madden and Morrison, 2005). The hypothesis that hypoxia inhibits sympathetically mediated thermogenesis in BAT was tested through hypoxic stimulation of arterial chemoreceptor afferents. This hypothesis was based upon the suggestion that a hypoxic induced reduction in hypothalamic temperature, brought about due to a hypoxia-induced hypothermia, may induce BAT thermogenesis. Accordingly an inhibition of BAT thermogenesis and BAT sympathetic nerve activity was observed in

rats during hypoxia. It is therefore suggested that inhibition of BAT sympathetic nerve activity at the peripheral chemoreceptors may contribute to hypoxia-induced reductions in body temperature. Based upon these findings, when exposed to an environment of high altitude and low ambient temperatures, the thermogenic response is blunted. It therefore seems reasonable to hypothesise that ambient temperature is of major importance with regard to the thermogenic response to hypoxia.

There is evidence to suggest that an inverse relationship exists between BAT activity and parameters of obesity (Dijk et al., 2015; Hao et al., 2014; Van Marken Lichtenbelt et al., 2009; Wang et al., 2011) and that activated BAT and beige adipose tissue improve systemic glucose homeostasis. This is based on the knowledge that active brown adipose tissue results in the combustion of lipids and glucose within the tissue (Cannon and Nedergaard 2004). In a study by Stanford et al., (2013) the hypothesised role of BAT in the regulation of glucose homeostasis was tested. Mice receiving transplanted BAT (0.1 g) from donor mice into the visceral cavity displayed significant decreases in body weight, improved glucose tolerance, increased insulin sensitivity coupled with a reversal of high fat diet-induced type II diabetes following eight to 12 weeks. In contrast, white adipose tissue is believed to contribute to chronic inflammation in obesity and disorders such as insulin resistance and type 2 diabetes through the secretion of adipokines (Lago et al., 2009). As a result a surge of interest in the function of BAT and the possible targeting of BAT for the treatment or the prevention of metabolic diseases has occurred. Specifically the activation of brown adipose tissue via cold exposure is increasingly scrutinised as a potential approach to ameliorate cardio-metabolic risk. (Dijk et al., 2015).

2.12.1 “BROWNING” OF WHITE ADIPOSE TISSUE

Recent studies have demonstrated that an increase in the presence of “brown-like” adipocytes can occur in subcutaneous white adipose tissue (Stanford et al., 2015). These “brown-like” adipocytes have been labelled a number of things including “adaptive brown fat cells”, “recruitable brown fat cells”, “beige cells” (Vegiopoulos et al., 2010), or “brite cells” (Shabalina et al., 2010). Some similarities between beige and brown fat exist with beige fat being characterised by the expression of UCP1. Perhaps the most important characteristic of these adipocytes is that they can derive from the subcutaneous white adipocyte depots through a process called “browning” (Wu et al., 2013). Browning of WAT can be achieved through several means including central nervous system activation which moderates sympathetic output to WAT (Warner and Mittag 2015). Browning can also occur through the recruitment and activation of immune cells in WAT, alternatively by direct action on white adipocytes or beige “precursor” cells (Warner and Mittag 2015). The process of WAT being converted to “beige” cells is displayed in Figure 2.11 (Ishibashi and Seale 2010).

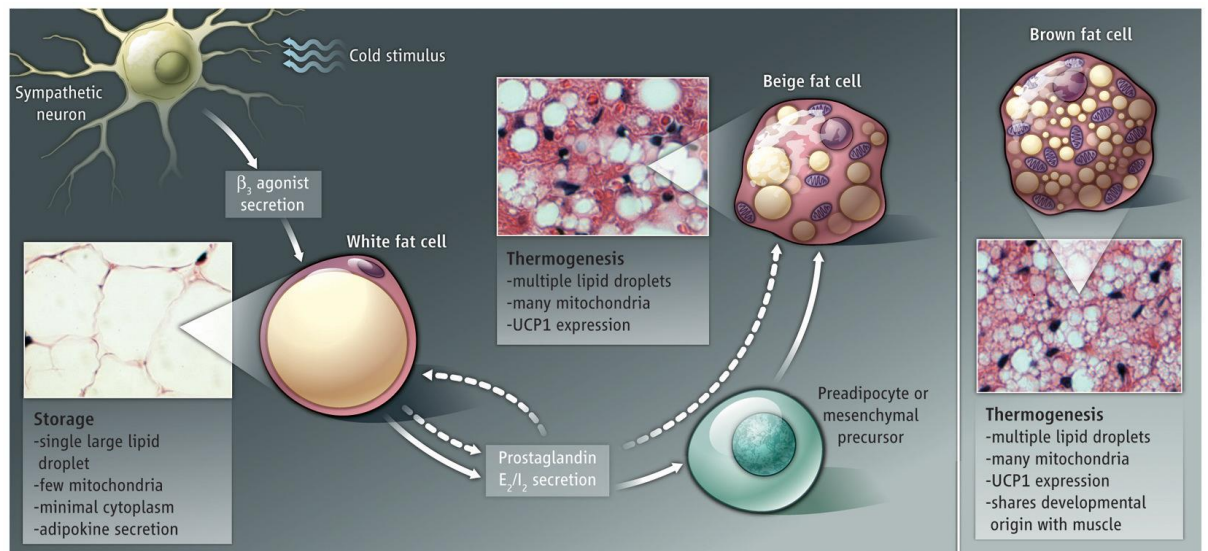


FIGURE 2.10: Diagram illustrating the process of white adipose tissue being converted into a “beige” adipocyte with descriptions of the thermogenic process (Ishibashi and Seale 2010)

Notes: Original in colour.

A number of “triggers” have been outlined for the “browning” of white adipose tissue including physical activity, cold exposure and a number of pharmaceutical agents. Regular physical activity is known to induce alterations in WAT; namely a decrease in cell size and reductions in lipid content resulting in decreased adiposity (Craig et al., 1981). Specifically a 12 week programme during which female rats were exercised five days a week for six hours a day induced reduced fat cell size in comparison to sedentary controls. Furthermore an increased number of insulin receptors and a six fold increase in the rate of glucose oxidation were observed in trained rats highlighting a greater responsiveness to insulin (Craig et al., 1981). An increased expression of UCP1, the marker of brown adipocytes, has also been shown following physical activity in the white fat of mice (During et al. 2012, Stanford et al., 2015). Specifically voluntary wheel running for as little as 11 days induced an increase in UCP1 and other genes indicative of brown or beige adipocytes in rodents with a greater increase in the subcutaneous depot in comparison to the visceral depot (Stanford et al., 2015). Such findings have led to the suggestion that the observed changes in WAT in response to regular physical activity are part of the process by which exercise improves whole-body metabolic health (Stanford et al., 2015).

Mechanistic explanations for the effect of physical activity on the “beiging” of adipose tissue are currently debated. It is suggested however that the exercise induced changes are induced by mechanisms that are different to the beiging brought about by cold exposure (Stanford et al., 2015). Beiging or browning through cold exposure is believed to occur due to increased heat loss which results in an increased thermogenic demand resulting in increased heat production (Nedergaard and Cannon 2014). One hypothesis for the exercise induced browning is that the exercised induced decrease in cell size and lipid content in subcutaneous WAT decreases insulation of the body

necessitating increased production of heat through “browning” of WAT (Nedergaard and Cannon 2014). Other suggestions include increased sympathetic innervation (Nedergaard and Cannon 2014) training-induced secretion of myokines (proteins secreted from skeletal muscle) (Pedersen and Febbraio 2012), and training induced secretion of hypothalamic brain-derived neurotrophic factor (During et al., 2012). Further work is required however in order to better understand the underlying physiological mechanisms leading to exercise induced browning.

There is however discrepancy between the thermogenic potential of beige adipocytes when studied in vitro compared to in vivo highlighting the possible limitations to the use of fat browning as an effective method of weight loss and obesity prevention/treatment. Specifically the same thermogenic potential has been shown in beige adipocytes as in brown when studied in vitro (Shabalina et al., 2013). When studied in rodents in vivo however it has been observed that beige fat demonstrates only approximately 20% of the UCP1- dependent oxygen consumption per gram of tissue compared to BAT (Shabalina et al., 2013). As such the meaningful contribution of recruited beige fat to whole body EE remains debated. One reason for the lack of consensus regarding the meaningful contribution of beige fat to EE is the lack of a direct measurement of its thermogenic contribution. This stems from the complexities of being able to single out the effect of beige fat in isolation from brown fat (Warner and Mittag 2015). As such greater research is required in order to understand in greater detail the effectiveness of beige fat in increasing EE in humans.

2.12.2 METRNL AND FAT “BROWNING”

A transcriptional coactivator that plays a role in the control of genes involved in oxidative metabolism and mitochondrial biogenesis is Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 α (Ruas et al., 2012). A highly expressed form of PGC-1 α termed PGC-1 α 4 is present in skeletal muscle in mice and humans particularly during exercise. The expression of PGC-1 α 4 in skeletal muscle stimulates increased mRNA expression and secretion of the hormone Meteorin-like (Rao et al., 2014). As such *Metrnl* has been identified as a key PGC-1 α 4 target gene and its release leads to the conversion of white adipose tissue to beige fat (Rao et al., 2014).

Meteorin-like protein is increased in mice following both a bout of concurrent (endurance and resistance exercise) and a bout of eccentric exercise highlighted by a 1.5 and a 3 fold increase in expression following resistance exercise and combined endurance and resistance exercise respectively (Rao et al., 2014). Interestingly *Metrnl* expression is also increased following acute cold exposure (six hours at 4°C and 24 hours at 4°C) but not chronic exposure (two weeks at 4°C) (Rao et al., 2014). In a separate study a long term intermittent cold exposure in which mice were exposed to 4 °C, two hours a day, five days a week for 14 weeks has been seen to improve glucose tolerance, enhance insulin sensitivity and reduce weights of epididymal and retroperitoneal adipose tissue in accordance with increased expressions of mitochondrial uncoupling protein (UCP1) and PGC1 α in subcutaneous adipose tissue (Wang and Sun, 2015). These findings suggest that cold exposure may

improve glucose homeostasis and induce white adipose tissue “browning” thus serving as a possible intervention to metabolic disorders.

Additionally to cold exposure and physical activity beneficial effects including increases in metabolic rate have been observed in obese, diabetic mice despite no change in physical activity and food intake or any observed alterations in RER following adenovirus induced overexpression of Metrnl (Rao et al., 2014). Specifically in transfected mice, a 25% reduction in whole body-fat content was reported following a 20-fold increase in liver Metrnl mRNA and a 5-6 fold increase in plasma Metrnl following three days. Meteorin-like protein, rather than having a direct action on adipocytes by promoting an increase in a thermogenic gene programme, appears to stimulate the expression of genes associated with beige fat thermogenesis and stimulate the immune cell subtypes to enter the adipose tissue, thus, activating pro-thermogenic actions (Rao et al., 2014). These findings support the notion that an increased mRNA expression and secretion of Metrnl may contribute to improved metabolic health. Whether similar responses are seen in humans however is unknown. Moreover it is also currently unknown whether exposure to other environmental extremes such as high altitude elicit similar responses to Metrnl and subsequent “browning” of adipocytes.

Exposure to environmental extremes including hot, cold and high altitude induce a number of physiological alterations in humans. Specific to high altitude and cold environments; despite the suggestion that differing, or perhaps even opposite physiological responses occur (Cadena and Tattersall, 2014) a number of similarities have also previously been outlined. As previously mentioned, a blunting effect of hypoxia to the thermogenic response in cold conditions exists (Blatteis & Lutherer 1976; Gautier et al. 1991; Cadena & Tattersall 2014). In a suggested attempt to protect against tissue damage upon initial exposure to hypoxia (Wood 1995) a decrease in oxygen consumption has been observed (Mortola, 1993). Furthermore, an apparent metabolic suppression and thus a reduced thermogenesis and a regulation of body temperature at a lower level is also reported in rats at $FiO_2:0.10$ (Mortola and Feher, 1998). In contrast however increases in metabolic rate at altitude have been reported (discussed in detail in section 2.7) upon acute (Butterfield et al., 1992; Mawson et al., 2000) and prolonged exposure (Westerterp et al. 1985; Westerterp and Klass 1994; Butterfield et al. 1992; Reynolds et al. 1999). In the first two days at an altitude of 4,300 m basal energy needs of men were elevated by as much as 40% above sea-level values before declining over a period of three to four days to stabilise at approximately 17% above sea-level values (Butterfield et al., 1992; Mawson et al., 2000). These latter findings are similar to the physiological responses to cold conditions. Increases in metabolic rate upon exposure to altitude may be driven by sympathetic activation (Butterfield et al. 1992; Mawson et al., 2000) and an induced higher basal noradrenalin levels (Urdampilleta et al., 2012). Other similarities highlighted between altitude and cold exposure include an initial diuresis and alterations in macronutrient effects, particularly the preferential use of carbohydrate (Brooks et al., 1991; Roberts et al., 1996). A description of both the similarities and dissimilarities between cold and altitude are highlighted by Askew (1995) in Table 2.3 and Figure 2.7.

Table 2.3: Similarities and dissimilarities between cold and high altitude (Askew 1995).

Similarities	Dissimilarities
Low ambient temperatures	Lower atmospheric oxygen tension at high altitude
Diuresis, at least initially	Usually greater anorexia and hypophagia at high altitude
Increased energy requirements for work	Fat tolerated well in the cold
Lack of water except for ice and snow	Fat not tolerated well at high altitude
Difficult to prepare food	
Carbohydrate is tolerated well	
Protein not particularly advantageous	

The similarities between cold and hypoxia allow for the existence of further parallels between the two stimuli to be hypothesised. Specifically within this thesis, the investigation, upon exposure to altitude, of the recently identified hormone Meteorin-like, is centred upon this suggestion. Increased mRNA expression and secretion of Meteorin-like is stimulated by the expression of PGC-1 α 4 (a novel form of the transcriptional coactivator PGC-1 α) in skeletal muscle. Skeletal muscle-specific transgenic over-expression of PGC-1 α 4 in mice induces muscle hypertrophy, increased basal EE and increased browning of white fat depots without changes in food intake or movement (Rao et al. 2014; Ruas et al. 2012). Meteorin-like is affected by stimuli including physical activity and cold exposure (Rao et al. 2014). With regards to environmental extremes; Metrnl expression is increased following acute cold exposure (six hours at 4°C and 24 hours at 4°C) but not chronic exposure (two weeks at 4°C) (Rao et al., 2014). Upon exposure to both high altitude and extreme cold an increase in sympathetic activation is observed which is a potential explanation for the observed increases in metabolic rate upon exposure to altitude (Butterfield et al. 1992; Mawson et al., 2000) along with induced higher basal noradrenalin levels (Urdampilleta et al., 2012). It may therefore be hypothesised that the effects of an altitude stay or exposure to hypoxia would raise Meteorin-like concentrations and may provide a mechanism for altitude induced reduction in fat mass. Increased sympathetic nervous system activity upon exposure to hypoxia/altitude may drive these increases. Similarities in findings, with regards to improvements in glucose tolerance, between mice delivered with Meteorin-like expressing adenoviral vectors (Rao et al., 2014) and obese individuals exposed to altitude (Schobersberger et al., 2003) highlight further rationale for an increase in Meteorin-like upon altitude exposure.

BROWN ADIPOSE TISSUE SUMMARY

Increasing the amount of brown adipose tissue has been shown to have advantageous effects on body composition and insulin sensitivity in mice. As such it is suggested that BAT is an endocrine organ that can function to improve whole body-tissue glucose homeostasis (Stanford et al., 2013) and may be a potential therapy for obesity in humans.

Cold exposure or beta-adrenergic activation of BAT causes lipolysis in brown adipocytes thus increasing the supply of fatty acids for oxidation. Glucose, free fatty acids and triglyceride-rich

lipoproteins present in the circulation are the major fuel sources for BAT and are subsequently taken up by brown adipocytes and contribute to the fuelling of thermogenesis (Dijk et al., 2015). Heat production by BAT is stimulated via release of noradrenaline (through B3 receptors) by the sympathetic nervous system.

The energy requirements of BAT increase “manifold” during cold exposure. These increased energy demands coincide with a marked increase in LPL activity thus stimulating the uptake of TRL-derived fatty acids (Bartelt et al., 2011). Less is known regarding the responses of BAT during exposure to other environmental stimuli including that of high altitude, although as previously discussed in this section the combination of cold and high altitude/hypoxia has received some attention (Blatteis & Lutherer 1976; Gautier et al. 1991; Madden & Morrison 2005; Cadena & Tattersall 2014).

The “browning” of WAT has been observed following cold exposure and regular physical activity in mice. This process has been highlighted as a possible method for increasing the thermogenic capacity of fat tissue in humans and thus increasing EE, reducing body mass and improving metabolic health. It is currently unknown however whether the contribution of beige fat to EE is of meaningful importance in humans in order to achieve these suggested beneficial effects.

Increases in the hormone *Metrn1* have been observed following cold exposure, physical activity and, through the delivery of *Metrn1*-expressing adenoviral vectors and such increases have been associated with an increase in EE associated with the browning of WAT depots. These findings suggest that intermittent exposure to cold may improve glucose homeostasis and induce white adipose tissue “browning” thus serving as a possible intervention to metabolic disorders. Less is known regarding the *Metrn1* response measured in humans however and whether exposure to other environmental extremes such as high altitude elicits similar beneficial effects.

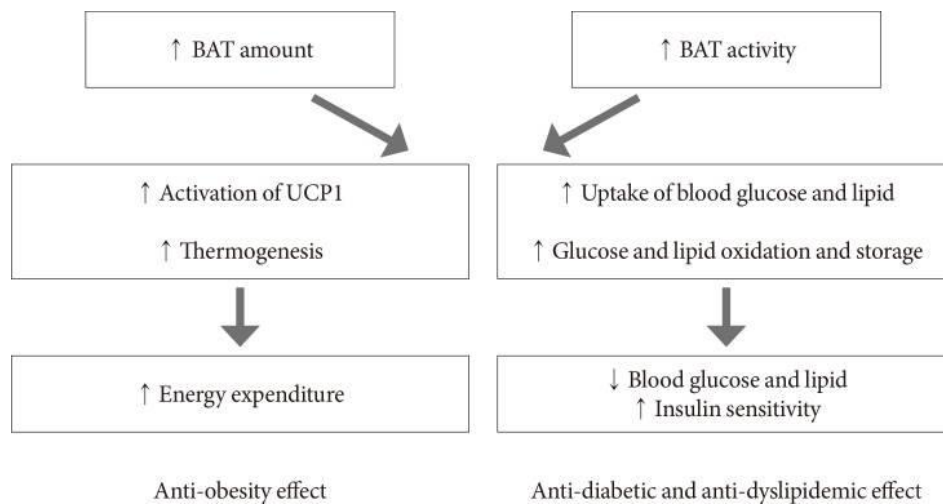


FIGURE 2.11: The suggested “anti-obesity” effects and “anti-diabetic and anti-dyslipidemic” effects of brown adipose tissue (BAT) (Kim and Plutzky 2016).

2.13 APPETITE REGULATION AND BODY MASS

OVERVIEW

Regulation of food intake is one of the most fundamental mechanisms for human survival and health. The worsening global epidemic of obesity has prompted research into the mechanisms of appetite regulation. Complex pathways modulate energy balance, involving appetite centres in the hypothalamus and brain stem, and hormonal signals released by the gut and the periphery (Druce and Bloom 2006). Appetite is also known to be regulated by the neural circuit for motivation and affection. A continuous use of energy occurs in humans; however the food intake that supplies that energy is episodic rather than continuous.

The storage of energy as glucose and glycogen is a mechanism to overcome the episodic nature of feeding and the continuous demand for energy. Most animals also store energy as fat; some do this in anticipation of a known future shortfall (e.g. hibernation) whereas other animals, such as humans, store fat in the absence of such anticipated needs; rather they do so in preparation for unpredictable shortfalls in supply. Storage of fat however brings both advantages and disadvantages and thus many animals have evolved to prevent them from storing too little or too much. The lower point on this scale is well established however the upper intervention point is less so. It has been suggested that human obesity could be potentially understood in an evolutionary context due to a drift in the upper intervention following release from predation, combined with a stimulus to overconsume calories (Speakman, 2014).

In overview a complex physiological system balances energy intake and expenditure, comprising of afferent signals and efferent effectors. Hunger leads to initiation of eating, when a meal is ingested,

satiety hormones contribute to digestion and a feeling of fullness. Central circuits in the brain integrate satiety signals and signals related to long term energy status to produce a response to the change in nutritional status (Druce and Bloom 2006). Exposure to environmental extremes including altitude, both real and simulated, evokes alterations in the appetite of humans as illustrated in Figure 2.13. The underlying causes for such changes however are not entirely known. The following section will initially outline the mechanistic explanations and the implication of appetite regulation in humans before summarising the available literature regarding exposure to conditions of reduced oxygen availability on appetite and subsequent food intake. The suggested causes for appetite alteration at altitude will also be explored.

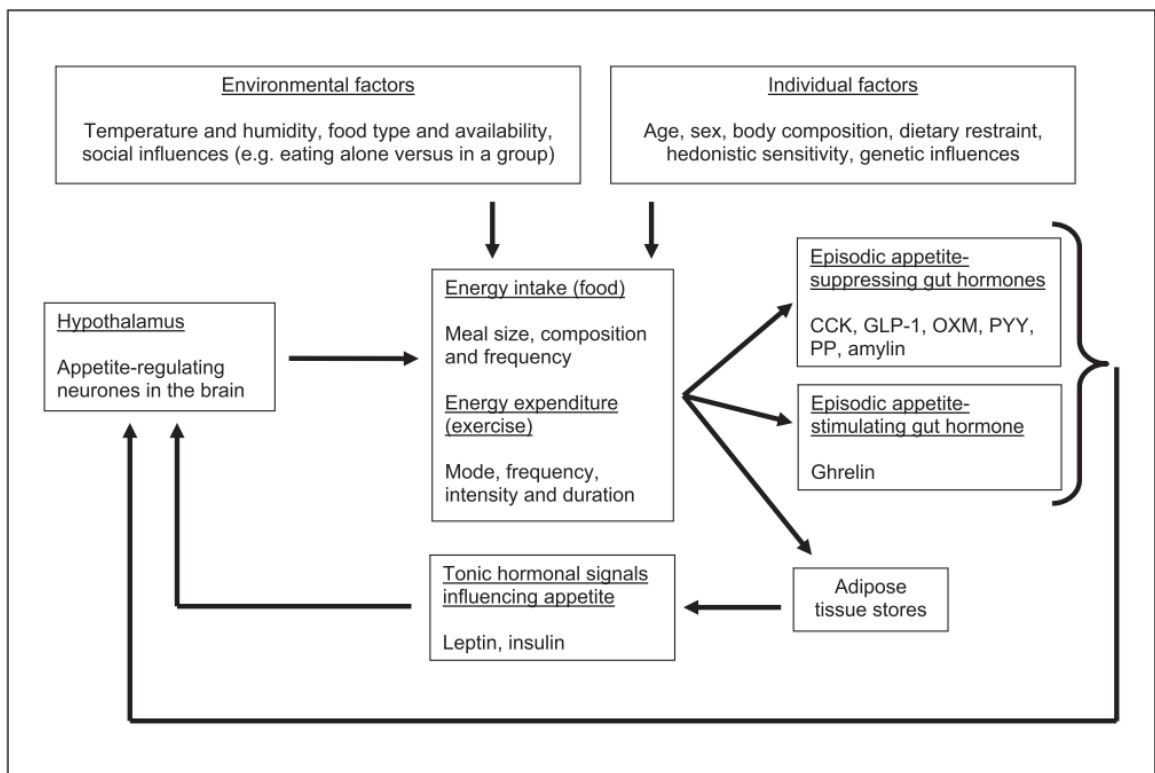


FIGURE 2.12: Overview of the major hormonal factors influencing energy balance and the possible interaction between exercise, environmental factors and energy intake (Stensel, 2011).

MAIN FINDINGS AND MECHANISTIC EXPLANATIONS

Intake of energy is adjusted in order to meet immediate changes in the demand. During intake of a meal a progressive decline in feelings of hunger or satiation is reported by humans. Neuroimaging has identified several regions in the human brain that are activated during food craving including the insula, the caudate, the hippocampus the inferior parietal lobe and the hypothalamus amongst others (Yan et al., 2011). In a study in which individuals with a thalamus infarct were investigated (Rousseaux et al., 1996) disorders on food intake have been observed highlighting the role of this area of the brain

in appetite regulation. The underpinning mechanisms for the regulation of satiation include direct distension of the gut, communicated directly to the brain via the vagus nerve, and the release of a number of hormones stimulated by digested nutrients. These hormones are released sequentially along the alimentary tract as digested food passes and is absorbed. These hormones, known as anorexic hormones, include cholecystokinin (CKK), glucagon-like peptide 1 (GLP1), vasoactive intestinal polypeptide (VIP), peptide YY (PYY), neurotensin (NT), oxyntomodulin (OXM), enterostatin. Apolipoprotein A-IV (APO), gastrin releasing peptide (GRP) and neuromedin B (NMB). The release of some of these hormones is mediated by the detection of digested nutrients by taste receptors and short chain fatty acid receptors on the luminal surfaces of L-cells in the alimentary tract which secrete the hormones (Jang et al., 2007). Different hormones differ in their responses to various nutrients that have been ingested. Furthermore differing hormones have separate target populations in the brain with the main target populations of neurons located in the brain stem (Speakman, 2014).

Many gut hormones including GLP1 and PYY feedback onto the gut, delaying motility and feed-forward to the pancreas and liver and adipose tissue in preparation for the absorption of nutrients. Appetite affecting hormones are also released from the liver, pancreas and adipose tissue including amylin, pancreatic polypeptide and insulin from the pancreas (Asakawa et al., 2003), adropin from the liver (Kumar et al. 2008) and many adipokines secreted from adipose tissue. These adipokines include Leptin and interleukin-6.

Satiety progressively declines after a meal is terminated until a reduction in this satiety stimulates the resumption of feeding. The reduction in satiety is determined by a combination of both a reduction in the aforementioned anorexic hormones and the release of the hormone Ghrelin produced by the stomach which acts in the hypothalamus as an appetite stimulant (Kojima et al., 1999). Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor and, in rodents, has been shown to be a potent stimulus to feeding with the maximum effect observed within an hour of administration and the resulting plasma levels comparable to that observed after a 24 hr fast (Wren et al., 2001) Chronic administration of Ghrelin has been shown to induce adiposity (Wren et al., 2001) and injection of anti-Ghrelin antibodies inhibits the normal feeding response (Nakazato et al., 2001). In humans Ghrelin plasma levels are high in a fasted state and fall after the ingestion of a meal (Cummings et al., 2002) moreover infusion of Ghrelin was seen to increase food intake by 28% compared to a control (Wren et al., 2001 2). It has been suggested that Ghrelin may play a role in the aetiology of human obesity. Ghrelin levels are lower in obese subjects compared to lean individuals with reductions in weight resulting in increases in levels of Ghrelin, which may result in difficulties in maintaining reduced body mass (Druce and Bloom 2006). Furthermore a contribution to overeating may derive from the observation that food fails to suppress Ghrelin levels in obese individuals (English et al., 2002) and thus may impair postprandial satiety.

PYY is produced by L-cells of the gastrointestinal tract and is released into the circulation after meals in proportion to the ingested calories. Administration of active PYY causes inhibition of food intake in both rodents (Halatchev et al., 2004) and humans (Batterham et al., 2002). Obese individuals have a lower fasting basal PYY level and a lower increase in levels after the ingestion of a meal however exogenous administration of PYY has an inhibitory effect on appetite (Batterham et al., 2003). GLP-1 is produced in the gut and brain by processing of the proglucagon gene and is released into the circulation after eating again in proportion to the amount of food consumed and acts on the pancreas to release insulin (Kreyman et al., 1987). Regulation of the secretion occurs through sensing the luminal concentration of lipids and carbohydrates (Schirra et al., 1996). In rodents peripheral and central administration of GLP-1 inhibits food intake (Wynne et al., 2005). In obese subjects GLP-1 secretion is reduced with normalising of the levels occurring with weight loss (Verdich et al., 2001). Interestingly the anorectic effects of GLP-1 are preserved in obesity (Naslund et al., 2004). Cholecystokinin (CCK) is released from the gastrointestinal tract after consumption of a meal. Release of CCK inhibits food intake via the brain stem in humans and rodents as well as stimulating gall bladder contraction, pancreatic secretion and gut motility (Beglinger, 2002). Also released postprandially in proportion to calories ingested is pancreatic polypeptide (PP) which is produced in the pancreatic islets and distal gut and suggested to play a role in appetite control (Adrian et al., 1976). Administration of PP has been shown to reduce food intake in normal weight individuals. Similar to GLP-1, Oxyntomodulin (Oxm) is produced by processing of preproglucagon in the gut and brain and is released after the ingestion of food in proportion to nutrient ingestion. Infusion of Oxm has been seen to reduce food intake during a buffet meal (Cohen et al., 2003) whilst chronic administration over one month resulted in significant losses of body mass of 2.3kg in overweight and obese individuals (Wynne et al., 2005).

Total intake of food is also sensitive to changes in the levels of EE, as increased energy demands induced by exercise (Kaiyala et al., 2012) or cold exposure (LeBlanc, 1996) stimulates food intake (Speakman 2014). It has also been suggested that the resting metabolic rate and the amount of lean mass (Blundell et al., 2012) of an individual has an effect on energy intake. In the short term the level of energy intake stimulated by the level of expenditure in conjunction with any shortfall being supplied by the mobilisation or storage of glucose and glycogen means that intake and expenditure may be balanced (Speakman, 2014).

In summary regarding the short term regulation of feeding behaviour, as outlined in Figure 2.14, when food enters the alimentary tract the production of several inhibitory compounds is stimulated. These compounds go to the brain stem and hypothalamus and inhibit production of the stimulatory compound Ghrelin which is produced in the stomach. These compounds also target additional hormone releasing peripheral tissues including the liver, pancreas and white adipose tissue which generate further inhibitory signals that also pass to the hypothalamus. Direct distension of the alimentary tract also acts as an inhibitory signal via the vagal nerve. The consequence of these

inhibitory signals combined with the reduced stimulatory signal is the decline in feelings of hunger and the eventual ceasing of feeding. Once feeding has stopped the inhibitory signals eventually decline and the stimulatory signals increase and food intake is eventually reinitiated.

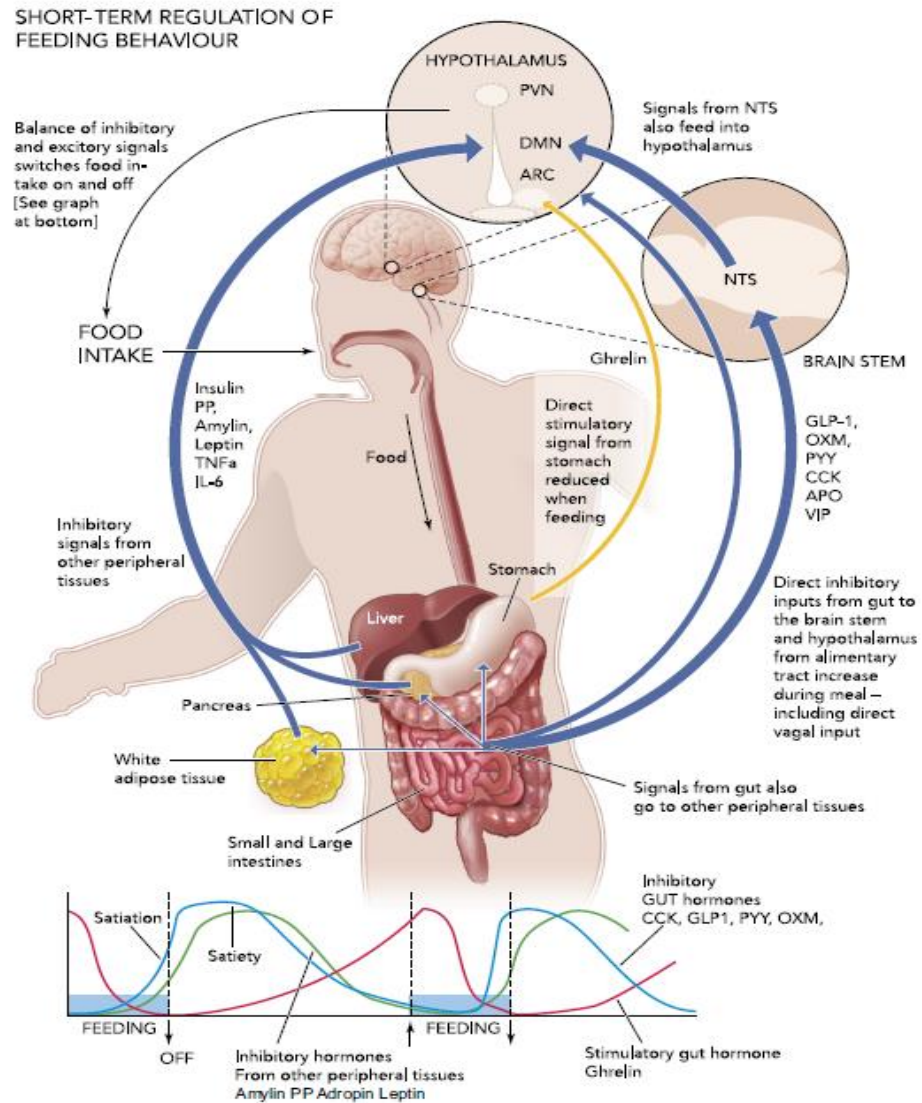


FIGURE 2.13: Short term regulation of feeding behaviour (Speakman, 2014).

Notes: Original in colour.

In the long term; storage of energy through fat and protein occurs in humans. During periods when food is abundantly available energy is stored as fat and protein so that it is available for periods in the future when demand will exceed supply. As humans cannot switch off their demands for energy and the reserves of glycogen and glucose are able to supply energy for only a limited period fat storage acts as protection against any possible shortfalls in energy supply.

One suggestion for the presence of obesity in humans is the thrifty gene hypothesis. Simply put this suggests that when there is a large amount of food available individuals respond by eating more and

depositing the excess stores as fat. During starvation those individuals with larger fat stores will survive for longer thus the thrifty gene hypothesis suggests that genes promoting fat storage will be more likely to be selected (Speakman, 2014). By this hypothesis the modern human obesity epidemic is caused by an environment where food supply is never constrained and the genes that promote fat storage have already been selected for. This idea however is questionable; one flaw with this hypothesis is that only a small amount of extra food is required in order to develop obesity over a period of time, furthermore in some wild animals there are situations in which food supply is not constrained at all yet obesity is not common amongst these animals. Furthermore for most humans in the Western society food supplies exceed energy requirements yet at the most obesity is only found in 30-35% individuals and around 20% remain lean. These flaws suggest that the thrifty gene hypothesis cannot fully explain the development of obesity in humans (Speakman, 2014).

The dual intervention point model suggests that there is a lower and upper intervention point in fatness and between these two points body fatness plays only a very little part in the regulation of food intake. Within these two intervention points it is suggested that social and psychological factors act in determining body fatness. If the level of fatness falls below the lower intervention point or above the high intervention point the model suggests that physiological mechanisms are enabled to stimulate intake or reduce intake in order to bring the level of fatness back within the two points (Speakman and O'Rahilly 2012). The physiological underpinning mechanisms for this regulating system have been examined extensively. The molecule Leptin is a major feature of such models. Leptin is a protein hormone produced by adipose tissue with regulatory effects on metabolism and body weight (Sierra-Johnson et al., 2008). The way in which Leptin is presumed to operate is through the interaction with receptors in the hypothalamus to inhibit eating (Sierra-Johnson et al., 2008). In the brain Leptin acts on hypothalamic neurons which express its receptors to modulate the expression of neuropeptides controlling feeding (Simler et al., 2006). Neuropeptide Y (NPY) and agouti-related protein (AgRP) participate in anabolic pathways by stimulating food intake. Conversely α -melanocyte-stimulating hormone (α -MSH) cleaved from proopiomelanocortin (POMC) and cocaine-amphetamine-related transcript (CART) inhibit food intake and promote negative energy balance. These peptides are regulated by Leptin which reduces anabolic and activates catabolic pathways. Low Leptin levels stimulate the NPY/ AgRP cells and inhibit the POMC/CART cells (Simler et al., 2006; Speakman, 2014). This system is often presented as a two- sided response in that low Leptin producing a stimulation of intake and reduction in expenditure, thereby elevating fat stores and high Leptin generating the reverse effects (Friedman and Halaas, 1998).

The ob/ob mouse is Leptin deficient, obese, hyperphagic and hyperinsulinaemic. With the administration of Leptin however this phenotype can be normalised (Robinson et al., 2000). The principal effect of Leptin is the reduction in hyperphagia. It is suggested that the Leptin model is more useful in relation to responding to low fatness and it seems likely that this control system only pertains effectively to stimulating intake to increase fat levels. The understanding of the mechanisms allied to

the upper regulatory point, i.e. what happens when fat levels are too high, remains relatively rudimentary. Furthermore the regulatory system appears not to be symmetrical and that although low Leptin levels may be an important signal for low fat levels and subsequently enable a number of mechanisms that stimulate intake and reduce EE, high Leptin levels, although correlated with body fatness does not appear to play a role that is as important for the upper intervention point of body fatness (Speakman, 2014). Defects in neuropeptide appetite circuits can deregulate energy homeostasis resulting in obesity. An example of such defects includes mutations of the POMC gene cause early onset of obesity (Krude et al., 1998).

SUMMARY

In summary regarding long term regulation of energy supply, the balance of energy is regulated from minutes to hours dependent on storage of glucose and glycogen to days and weeks related to storage of fat. The regulation of body fat centres around the prevention of body stores becoming too low dictated by the risk of starvation and prevention of the body stores becoming too high, once dictated by the risk of predation. The obesity epidemic can be understood, it is suggested by Speakman (2014), through a genetic drift in the upper risk of obesity stores due to a reduction in the risk of predation in humans in the last 2 million years. Despite this relatively little is known regarding the underpinning physiological mechanisms of the upper level of obesity stores.

2.14 ANOREXIA AT ALTITUDE/ HYPOXIA

OVERVIEW

Appetite suppression and reduced food intake has been reported at altitude in both field (Bailey et al., 2000; Barnholt et al., 2006; Kalson et al., 2010; Westerterp et al., 2001) and laboratory (Westerterp-Plantenga et al., 1999) studies. Decreased appetite and energy intake collectively termed “altitude anorexia” is frequently reported during high altitude exposures and can induce negative energy balance especially when coupled with increased levels of physical activity. The overall picture regarding appetite at high altitude has been described as complex (Kayser and Verges 2013) with a reduction of 200 kcal.day⁻¹ approximated from previous work at 4,300m (Butterfield et al., 1992). Similarly energy intakes have been reported to decrease by between 17 and 57% at altitudes ranging from 3,600m to 6,000m (Wasse et al., 2012). Furthermore it is suggested that 20% of body mass loss experienced over an eight day period at 4,300m can be explained by a voluntary decrease in caloric intake (Surks et al., 1966).

Altitude-related appetite modulation is not entirely understood and initially the observed appetite reduction was only linked to acute mountain sickness (Gallagher and Hackett 2004) however the reported reductions in appetite, as well as energy intake after the subsiding of AMS symptoms imply alternative underlying mechanisms. It had previously been suggested that lack of food availability and

palatability was a major cause for reduced intake whilst at high altitude and thus presumed contributors to cachexia (Palmer and Clegg 2014).

Losses of appetite observed in studies in which hypoxic chambers are used however suggest that it is hypoxia per se that causes the altitude related loss of appetite (Westerterp-Plantenga, 1999). Furthermore chamber studies that have offered a variety of palatable foods and encouraged food consumption demonstrate a loss of body mass due to a reduction in energy intake further highlighting the effect of hypoxia (Butterfield et al., 1992; Westerterp-Plantenga, 1999). Loss of appetite at altitude has primarily been addressed through the monitoring of food intake. It is proposed from Butterfield et al., (1996) that sea-level and altitude energy intakes are closely correlated as shown in Figure 2.15. This is based upon studies in which reliable information is available for both sea-level and altitude food consumption and suggests a reduction in approximately 180 kcal.day⁻¹ at altitude.

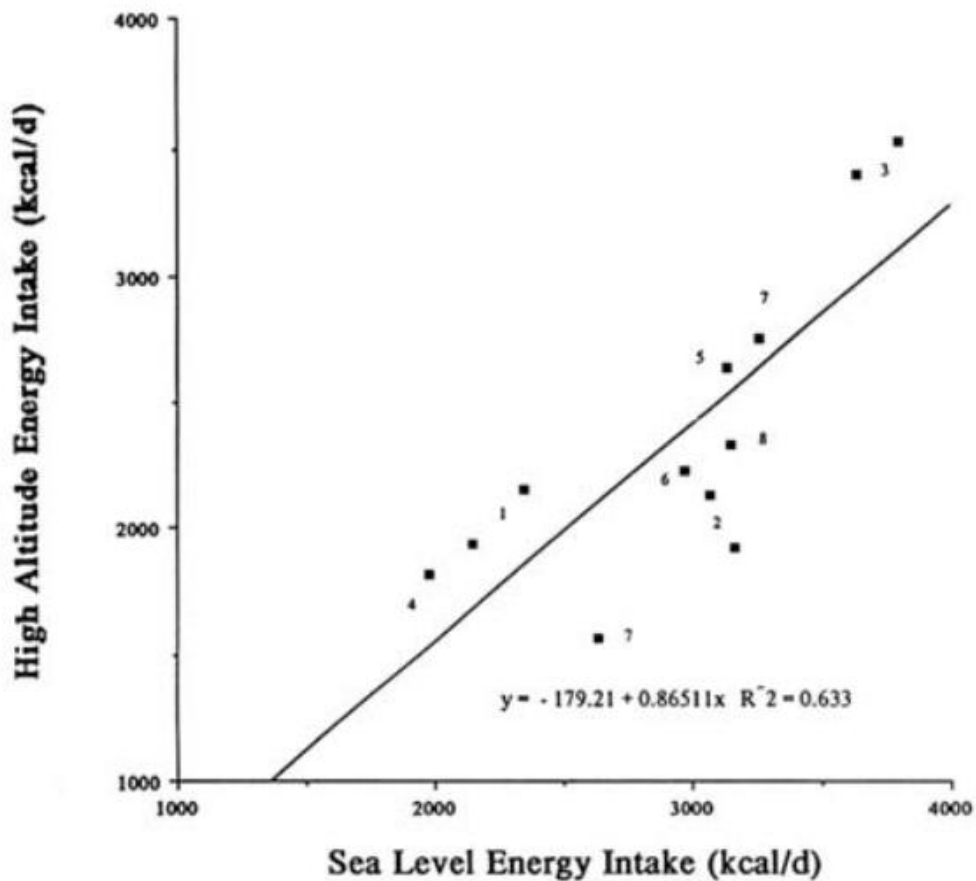


FIGURE 2.14: Relationship between sea-level energy intake and high-altitude energy intake (kcal.day⁻¹) compiled from studies with ad libitum food intake (Marriott and Carlson, 1996).

MAIN FINDINGS AND MECHANISTIC EXPLANATIONS

One explanation for an alteration in appetite upon altitude exposure is an increase in blood noradrenaline concentration which have been observed to triple on reaching a pressure of 282 Torr

following a simulated ascent of Everest (Young et al., 1989). Increased sympathetic activity during ascent would be expected to alter gut blood flow because of increased intestinal sympathetic tone, which has been suggested as a reason for a reduction in appetite (Loshbaugh et al., 2006). However in a study in which a loss of appetite was reported at an altitude of 4392 m increased resting blood flow and a maintenance of increased post-prandial blood flow was reported thus suggesting that reduced flow is unlikely to cause reduced appetite at high altitude (Kalson et al., 2010).

Interactions between the gastrointestinal system, adipose and muscle tissue and the central nervous system combine in order for food ingestion behaviour and energy intake to reflect the varying energy needs of a given situation or environment. The role of appetite regulating hormones at altitude is not well established. Hormonal regulation may potentially play a role in the alteration of appetite at altitude as several appetite-related gut hormones and adipokines are affected by acute and or prolonged exposure (Kayser and Verges 2013). The release of adipose tissue hormones such as Leptin and Ghrelin may mediate alterations in feeding (Scherer, 2006; Smith et al., 2011). Leptin is a protein hormone produced by adipose tissue with regulatory effects on satiety, metabolism and body weight (Sierra-Johnson et al., 2008). Exposure to hypoxic conditions has been shown to stimulate an important regulator for the expression of hypoxic inducible factor resulting in the release of Leptin (Sierra-Johnson et al., 2008). It has been suggested that Leptin may play a role in high altitude anorexia. The effect of altitude on circulating Leptin levels in humans however is equivocal and controversial (Shukla et al., 2005; Tschöp et al., 1998) with increases (Shukla et al., 2005; Tschöp et al., 1998), and decreases or no changes in the measure (Chaiban et al. 2008; Barnholt et al. 2006; Vats et al. 2004) presented following exposure.

Suggested explanations for the differences in Leptin response to altitude between the aforementioned studies centre upon confounding factors often associated with altitude exposure such as physical exertion during ascent and single measures of Leptin compared to multiple measures (Tschöp et al. 1998). Furthermore ambient temperature, diet and genetic adaptation of the participants may also contribute to an alteration in the altitude/Leptin relationship (Sierra-Johnson et al., 2007). Specifically it is suggested that hypoxia directly stimulates Leptin release under controlled experimental conditions however Leptin may decrease due to the aforementioned confounders associated with altitude (Snyder et al. 2009). It may therefore be suggested that laboratory based studies are more likely to result in increases in plasma Leptin compared to field bases studies during which more potential confounders are harder to control for and therefore present.

Work from Tschop et al., (1998) investigated the effect of hypobaric hypoxia at high altitude on serum Leptin concentrations. Studies in which increases in Leptin have been reported at altitude include an increase in Leptin concentrations following 22 hours at 4,559m and this effect was irreversible after 1 hour of treatment with 33% oxygen enriched air. Furthermore the observed increases in Leptin were apparently more pronounced in those individuals reporting a loss of appetite documented by the

Environmental Symptom Questionnaire compared to those expressing no appetite loss (Sampson et al., 1983). In light of these results however it was suggested that the exertion of the individuals may be a confounding factor as the release of Leptin is a pulsatile process. In an attempt to account for exertion a follow up study measured 18 individuals for serum Leptin at both 490m and at 4559m following transport by helicopter. In individuals with loss of appetite mean serum Leptin increased with no increases observed in those individuals without loss of appetite (Tschop et al., 1998).

It has been suggested, that all factors usually associated with high altitude such as cold, limited food supplies and overexertion should be considered when assessing the Leptin/ altitude relationship (Sierra-Johnson et al., 2007). This is the case as such factors may decrease levels of Leptin thus blunting or hiding the effect of altitude on circulating levels resulting in a misinterpretation of the effect of altitude alone (Snyder et al. 2009). High altitude exposure also increases the expression of a variety of genes including Leptin gene promoter, resulting in an increase in its level as well as increasing insulin messenger RNA (Chaiban et al., 2008). It has been stated that insulin and hypoxia work synergistically to induce Leptin transcription (Meißner et al., 2003) and that there is correlation in previous studies with increases in Leptin and increases in altitude, loss of body mass and loss of appetite. The mechanistic role of Leptin at altitude however is uncertain and it is suggested that Leptin may not be the main contributor to hypoxic-induced anorexia (Quintero et al., 2010). This is supported by a study which demonstrated a reduction in calorie intake in Leptin-receptor deficient mice following exposure to hypoxic conditions in hypobaric chambers progressively reduced to an equivalent of 5,500m lasting for up to four days (Simler et al., 2006).

Acute hypoxia has been shown to reduce circulating levels of the appetite stimulating hormone Ghrelin, both when individuals are fasted (Shukla et al., 2005) and fed (Wasse et al., 2012). The majority of the literature indicates that levels of total Ghrelin decrease at high altitude in the short term (≤ 2 days) (Shukla et al., 2005) but are unchanged in the long term (seven days to seven weeks) (Benso et al., 2007; Shukla et al., 2005). An initial reduction in Ghrelin was reported at high altitude following ascent to 4,300m (Shukla et al., 2005). This was found in conjunction with increased Leptin concentration and a reduction in both energy intake ($850\text{kcal}\cdot\text{day}^{-1}$) and body mass. Recent work from Wasse et al., (2012) reported suppressed hunger and energy intake in conjunction with suppressed values of acylated Ghrelin during a 7 hour stimulated hypoxic exposure of 7,000m. Furthermore work from Broom et al., (2006) reported suppressed acylated Ghrelin following exercise alone. The response of the satiating gut hormone glucagon-like peptide-1 has been less well researched thus far although a trend for increased values following an overnight exposure to a simulated hypoxic environment equivalent to 4,100m has been reported (Snyder et al., 2009) further highlighting the influence of altitude on appetite regulating gut hormones.

Similarly to hypoxia, acute or chronic exercise significantly alters hormonal appetite regulation (Schubert et al., 2014). Acute exercise transiently suppresses appetite whilst chronic exercise training

typically results in an augmented appetite leading to a restoration of the exercise induced energy imbalance (Stensel, 2011). For example it has been demonstrated that six weeks of regular aerobic exercise training improves appetite regulation in previously inactive men and women through facilitation of more sensitive eating behaviour in response to previous energy intake so that individuals down-regulate energy intake after a high energy preload compared with a low energy preload (Martins et al., 2007). Furthermore there is evidence to suggest that a sedentary lifestyle predisposes to a failure in appetite regulation whereby energy intake is uncoupled from EE (Murgatroyd et al., 1999) suggesting a link between inactivity and disrupted homeostatic mechanisms involved in appetite. Energy deficits induced by exercise induce different effects compared to those induced by diet it has been reported (Martins et al., 2007). Although the majority of studies show little or no effect following an acute bout of exercise on feelings of appetite and satiety physically active individuals such as long distance runners usually have a greater energy intake than sedentary individuals but also usually have a lower BMI suggesting a tight coupling between energy intake and expenditure at high levels of physical activity (Martins et al., 2007).

Exact mechanisms underpinning the effects of exercise on appetite are however still unclear, although reductions in PYY and GLP-1 have been outlined as possible key factors (Stensel, 2011). The investigation of the acute effects of exercise on food intake also poses a number of difficulties similarly to all studies in which food intake is monitored and/or recorded. These difficulties include individuals altering their food intake when being monitored or, if self-recording their diet, the under reporting of food intake is also a common issue (Stensel, 2011). Most commonly used to assess the effects of exercise on food intake are the ad libitum buffet meals which have been shown to be reproducible by at least one study (Gregersen et al., 2008). Although effects of exercise on appetite have been observed from some studies in a review regarding exercise, appetite and food intake Stensel (2011) reported that the majority of studies report no change in energy intake following an acute bout of exercise with only 19% of studies employing buffet meals reporting an increase in intake, 65% showing no change and 16% showing a decrease in uptake (Blundell and King 2008).

The effect of exercise training rather than an acute bout of activity on appetite, food intake and weight control has also been somewhat investigated. Increases in concentrations of PYY have been reported following training programmes of varying lengths highlighting the role of exercise training on increased satiety after weight and fat loss. Work from (Jones et al., 2009) observed a significant increase in PYY concentrations following a 32 week training programme in individuals who were overweight in conjunction with a loss of body fat. Similarly (Roth et al., 2005) reported greater PYY concentrations in obese children who lost body mass following a 12 month programme and (Martins et al., 2010) observed a trend for higher postprandial PYY concentrations in overweight and obese individuals following a 12 week intervention. In summary there is evidence that exercise can have a beneficial influence on appetite and appetite regulating hormones facilitating a negative energy balance and weight loss (Stensel, 2011). Generally speaking increased physical activity has been seen

to cause a partial increase in energy intake whilst reducing exercise will not necessarily translate into reduced energy intake. It is however important to recognise the effect of age, sex, body composition, physical activity levels, individual differences and environmental factors such as altitude on the effect of exercise on appetite and energy intake.

The combination of exercise and hypoxic exposure has most commonly been used as a method to improve endurance performance; however this combination has recently been increasingly used in an attempt to combat obesity and its related comorbidities. A recent study from Bailey et al., (2015) investigated the effects of continuous moderate intensity exercise versus high intensity interval exercise in combination with short exposure to hypoxia on appetite and plasma concentrations of acylated Ghrelin, PYY and GLP-1. It was suggested from this study that appetite perceptions and plasma acylated Ghrelin may be suppressed in response to as little as 50 minute's normobaric hypoxic exposure whilst performing exercise although the modality of exercise did not appear to be an influencing factor. Concentrations of GLP-1 and PYY were unaffected by short exposure to hypoxia combined with exercise in this study.

The effects of longer term hypoxic exposure and exercise have also been investigated regarding hormonal appetite regulation. In one of the early studies investigating the combined effect of hypoxia and exercise 10 days of normobaric hypoxic confinement coupled with either daily moderate intensity exercise or no exercise were used to determine the acute and prolonged effects on measures of Ghrelin, PYY, coupled with perceived appetite scales and metabolic markers including body mass, body composition and insulin sensitivity (Debevec et al., 2014). Average energy intake compared to pre-values was lower in the exercise group alone during the hypoxic confinement, however contrary to the studies initial hypothesis the addition of exercise to the 10 day exposure did not significantly affect hormonal appetite regulation in fasted and postprandial states. Fasting levels of Ghrelin, Leptin, GLP-1 and PYY in the study were unchanged following both acute and chronic exposure to a simulated altitude of 4,000m as were appetite ratings between exercising and non-exercising groups and from pre-values to hypoxic values. Body mass was significantly reduced in both groups following the 10 day exposure although a reduction in fat mass was seen only in the exercise group. The combined findings of reduced energy intake and unchanged appetite perceptions could suggest a reduced motivation to eat during combined hypoxic exposure and exercise. A lack of change in perceived appetite coupled with a lack of change in appetite regulating hormones however could also be suggestive of a lack of change brought about by either stimulus and the stimuli combined. Further research is required in order to determine causality of hypoxic exposure on regulating hormones such as Ghrelin and to determine causality of these hormones on suppressed appetite.

In a similar study (Morishima et al., 2014a) the effect of normobaric hypoxic training on metabolic syndrome related factors particularly regional fat accumulation was conducted in conjunction with investigating the changes over time in appetite related hormones following the consumption of a

meal. The study hypothesised that the combination of hypoxia and exercise would; promote a further reduction in visceral fat area, improve insulin sensitivity and improve the regulation of orexigenic and anorexigenic hormones. The main finding from the study in relation to appetite however was no change in appetite-related hormonal response following the hypoxic training. Fasting and postprandial plasma Ghrelin concentrations were similar before and after the training period and postprandial area under the curve measures of Leptin significantly decreased in both normoxic and hypoxic training groups. Increases in fasting and postprandial plasma GLP-1 concentrations were recorded following normoxic training whereas no change was seen in the hypoxic group. The observed increase in GLP-1 was seen in conjunction with an attenuated score of hunger suggesting that exercise training may have a role in attenuating appetite in a fasted and postprandial state. The hypoxic training did however observe a greater improvement in postprandial glucose tolerance compared with normoxic training with no loss of whole body fat mass although the conclusion drawn from the work was that short term hypoxic training did not alter appetite related hormones at fasted as well as postprandial states.

The effect of prolonged high altitude residency on neural and gut processing and food craving has been investigated in individuals whom had been residents at high altitude (2,616-4,200m) for more than twenty years by obtaining functional magnetic resonance imaging (fMRI) data whilst viewing food pictures (Yan et al., 2011). The authors hypothesised that those who had been altitude residents would display decreased brain activity compared to sea level controls. In agreement with the hypothesis and in apparent agreement with decreased appetite with high altitude residence high altitude residents showed decreased activation in a number of regions of the brain including the insula and visual cortex whilst viewing images of food. The high altitude group also showed no activity in the thalamus which was not the case for the sea-level controls thus prompting the authors to conclude that high altitude residence may induce decreased activation in the neural circuit for food craving and cognitive control.

In summary altitude exposure can depress appetite, resulting in significant energy deficit. Food availability, palatability or both may also be compromised at altitude which in turn may exacerbate this situation. Furthermore each task is relatively more difficult at altitude increasing the shift in fuel utilisation to carbohydrate derived sources but the normal shift towards muscle glycogen and blood glucose during exercise could be limited at altitude by the availability of fuel sources. Forced catabolism of lipid and even lean tissue yields a smaller amount of fuel per unit of O₂ consumed and is less preferred by working muscle. It is therefore suggested for individuals at altitude to place emphasis on consuming sufficient carbohydrate rich foods.

SUMMARY

Energy balance and eating behaviour in humans is regulated by a complex system involving appetite centres and hormonal signals. An episodic system of energy intake is responsible for the continuous

use of energy. Energy storage in humans is therefore pivotal. Short-term storage is apparent in the form of glucose and glycogen whereas long term storage of energy is present in the form of fat and protein. Several contributing factors exist with regards to the regulation of appetite and energy intake. Such factors include, amongst others, EE, physical activity, and exposure to environmental extremes.

With the increasing incidence of obesity there is increasing research regarding the control and regulation of appetite, energy intake and subsequent effects on body mass. A number of theories exist regarding the development of obesity in humans despite a regulated energy balance system including the “Thrifty Gene Hypothesis” and the “Dual intervention point” theory. Although these theories highlight important contributing factors no one explanation is considered an all-encompassing explanation.

Exposure to an environment of reduced oxygen availability has been observed to reduce appetite and food intake in humans. Such findings are reported at real and simulated conditions. Specifically an altitude induced reduction of approximately 200 kcal.day⁻¹ has been observed in males at 4,300m highlighting the significant contribution of eating behaviours above sea-level. Furthermore findings from chamber studies in which food availability and palatability are controlled for suggest that hypoxia per se is likely to have a contributing effect to the observed reduction in energy intake. It has been suggested that weight loss at altitude may be overcome through enforced eating of palatable and easily consumed foods (Butterfield et al., 1996) although this is debated particularly at high altitude (Macdonald et al., 2009).

Suggestions as to the underlying mechanisms for reduced appetite at altitude have in the past included reduced intake being a response to AMS however more recently suggestions including disturbances in appetite controlling hormones such as Leptin, Ghrelin, PYY, GLP-1 and others are presented. Furthermore reductions in the neural processing of appetite following residence at high altitude resulting in lowered craving of food is also hypothesised. Future work is required to further understand the interplay between exposure to an environment of low oxygen availability and the response of humans with regards to appetite and food intake.

Regulation of food intake, feelings of satiety and palatability are regulated, in part by taste. Accordingly weight loss has been described as a direct consequence of reduced or lost sensations of taste (Woschnagg et al., 2002). Potential reasons for this effect include an increased monotony of flavour and thus, a reduced palatability and pleasantness of food culminating in earlier satiety and reduced energy intake. This is supported by work in which the repeated presentation of some foods can lead to a persistent decrease in the pleasantness of the presented foods (Schutz and Pilgrim 1958; Siegel and Pilgrim 1958) and furthermore the consumption of a monotonous liquid diet, was found to cause subjects to voluntarily restrict their energy intake and thus lose weight (Cabanac and Rabe 1976).

Although evidence regarding alterations in taste upon exposure to altitude is limited, some work has been completed which informs the rationale for investigation within this thesis (Singh et al., 1997, 1996). Humans exposed to 3,500m for a period of three weeks demonstrated changes in thresholds for a number of differing tastes. Specifically; increases in taste thresholds for glucose and sodium chloride and decreases in taste thresholds for quinine sulphate and citric acid were observed, all of which showed a tendency to return to baseline upon return to sea-level. Similarly in a rat model the effect of a three week exposure to hypoxia corresponding to an altitude of 7,620m observed a preference for sweet tasting solutions during exposure that returned to pre-exposure levels upon restoration of normoxia (Singh et al., 1996). These latter findings highlight a potential alteration in food intake at high altitude based on an increased importance of sensory cues such as preference for sweet flavour. Based on these findings it is suggested that hypoxic stress increases the palatability for sweetness which may be caused by an anorexia linked stress (Singh et al., 1997). Accordingly, correlations exist between taste perception and anorexia (Ames et al., 1993; Dewys and Walters, 1975; Mattes and Cowart, 1994). Cravings of sweet, salt, and bitter tastes have, however, been attenuated during high altitude residency (2,616 - 4,200 m) (Yan et al., 2011) highlight potential conflicting results. These previous findings provide support for the investigation of potential changes in taste sensation upon exposure to altitude as a potential cause for changes in eating habits and thus energy intake and body mass.

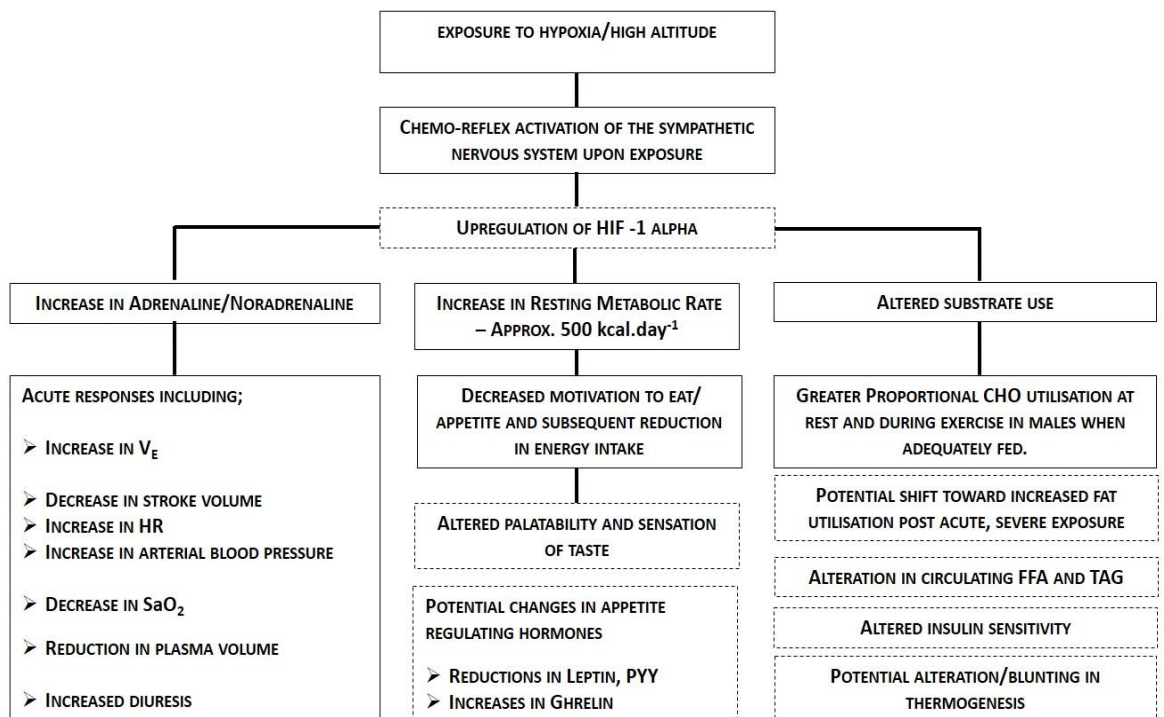


Figure 2.15: A summary of the metabolic responses to hypoxia/ high altitude.

Notes: Solid line boxes indicate responses that are supported by previous research. Dashed boxes indicate responses that, although have supporting evidence, are less examined.

2.15 THE ROLE OF EXERCISE FOR LOSS OF BODY MASS AND CHANGES IN CARDIO-METABOLIC HEALTH

OVERVIEW

Physical activity can be defined as any bodily movement produced by skeletal muscle that results in EE beyond resting EE (Feo, 2013). Physical activity has long been associated with many health-related benefits including a reduced risk of developing several chronic diseases and a lower risk of premature mortality (Warburton et al., 2006). The beneficial effects of physical activity has prompted the development of guidelines which were initially designed to improve the health of an already “healthy-weight” population and for the purposes of preventing the onset and increased risk of disease (Katzmarzyk and Lear 2012). Exercise is defined as a subset of physical activity that is planned, structured, repetitive and purposeful with the maintenance of physical fitness being the objective (Colberg et al., 2010). The relatively recent increase in prevalence of overweight and obesity has prompted the development of guidelines for both physical activity and exercise for a different purpose. This is to effectively implement guidelines that will result in the loss of body weight and body fat for those who require a reduction to improve health and reduce their risk of premature mortality and chronic diseases.

PREVIOUS FINDINGS AND MECHANISTIC EXPLANATIONS

Energy balance has been outlined as a key concept for the loss and maintenance of body mass. Weight reduction reduces the health risks associated with chronic diseases and a loss of 10% in weight for overweight and obese people is encouraged in order for these benefits to be observed (Donnelly et al., 2009). Restriction of energy intake in the long term though is difficult to sustain and generally results in the gaining of weight over time. In contrast regular physical activity and exercise has been associated with successful management of body weight as it provides a means for increasing EE (Donnelly et al., 2004) thus helping to sustainably adjust the energy balance. Evidence demonstrating only moderate effects of exercise on body mass however suggests that the true role of exercise in weight management remains relatively poorly understood (Colley et al., 2010) and that the need for further investigation is still present.

Research into the effects of exercise on weight loss has been prompted, through the increased prevalence of obesity. The effect of physical activity of varying modes, durations and in differing environments, on its effectiveness in inducing a loss of body mass is now extensive. It is suggested that exercise has an appropriate dosage in relation to the required goals and in a systematic review it is observed that there is accumulated, consistent evidence suggesting that physical activity is an effective strategy to prevent and treat obesity and its co-morbidities (Vasconcellos et al., 2014). The American College of Sports Medicine (2009) concluded that moderate intensity physical activity of 150 to 250 minutes per week with an energy equivalent of 1,200 – 2,000 kcal per week was sufficient to prevent weight gain greater than 3% in most adults and may result in modest weight loss. Physical

activity of > 250 minute week however of the same intensity has been associated with clinically significant weight loss and improved weight maintenance following weight loss (Donnelly et al., 2009). Furthermore it was observed that physical activity without restriction on diet generally provides modest weight loss whereas the additive effect of diet and physical activity provides an increased weight loss compared to diet alone (Donnelly et al., 2009). Moreover for exercise to effectively contribute to weight management it must occur in addition to baseline levels of physical activity (Colley et al., 2010).

Effective methods specific in inducing weight loss have been described exhaustively within the literature with both continuous moderate intensity (Tjonna et al., 2008) and high-intensity (Tjonna et al., 2009) forms of exercise having been shown to be useful in overweight and obese individuals. However presently within the literature there is little conclusive evidence to suggest that more favourable effects are apparent when using high intensity exercise training compared to continuous moderate-intensity exercise (Feo, 2013). In comparison to resistance training alone; aerobic forms of exercise are deemed more efficient for weight loss (Willis et al., 2012).

The effect of exercise intensity on substrate utilisation and thus weight loss has been established through the availability of stable isotope techniques, nuclear magnetic resonance spectroscopy, indirect calorimetry and tissue biopsy. These techniques used within a multitude of studies have allowed for the investigation of the contribution of glucose and FFA to ATP in exercising humans. It is clear that energy flux is determined by relative exercise intensity (Brooks, 1998). When at rest, around 60% of energy for non-contracting skeletal muscle is derived from lipid oxidation (Brooks, 1998). During moderate intensity exercise both lipid and glucose are equally oxidised by working muscles. As exercise intensity increases carbohydrate becomes the primary energy source and FFA flux and oxidation are inversely related to exercise intensity (Feo, 2013) and at particularly high exercise intensities of above 80%, the utilisation of FFA declines below basal levels at which point the energy supply of working skeletal muscle becomes dependent on muscle glycogen and blood glucose (Brooks and Mercier, 1994) (Figure 2.16).

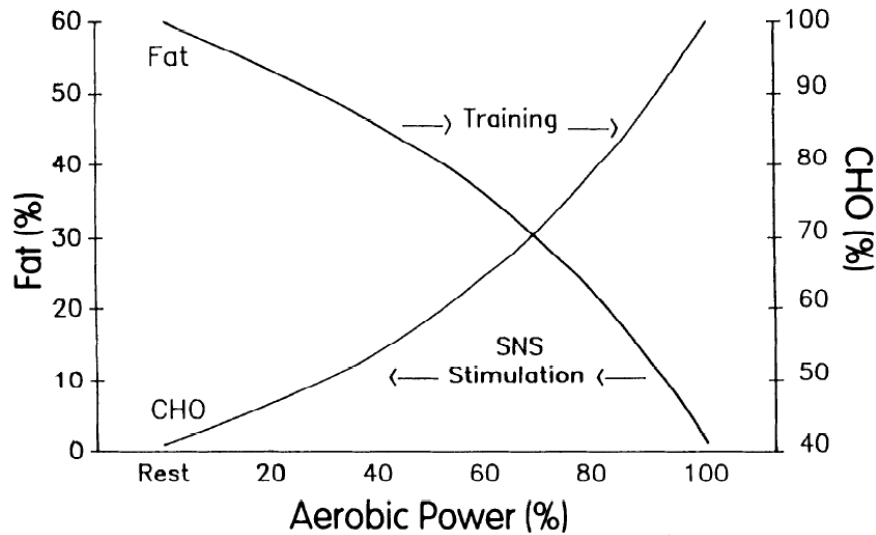


FIGURE 2.16: Relative increase in energy derived from carbohydrate (CHO) utilisation and decline in energy from oxidation of lipid utilisation as a function of relative aerobic power. At the point of crossover increments in relative exercise intensity results in an increasingly greater dependence on CHO and less dependence on fat (Brooks et al., 1994).

Notes: Sympathetic nervous system (SNS).

The depletion of muscle glycogen due to high intensity exercise elicits greater lipid oxidation rates in the 24 hour period following intense exercise in comparison to moderate exercise which can have implications for exercise mediated fat loss and in theory high intensity exercise could allow patients to lose more fat mass than moderate intensity exercise considering that it induces increased oxidation of lipids after the session (Feo, 2013). For numerous reasons however there is little conclusive evidence currently available suggesting that high intensity exercise is more beneficial than moderate intensity exercise for loss of fat and body mass. As both moderate and high intensity exercise has been seen to induce losses of body mass it is suggested that the decision surrounding exercise intensity may be best made individually and on differences on preference/ need for losses of body weight versus losses of body fat (Feo, 2013).

A variety of mechanisms by which exercise can alter energy balance have been outlined and are commonly separated by the energy expended during the exercise activity, energy expended shortly after the activity and the exercise induced alteration in resting metabolism (Donnelly et al., 2004). It has been suggested that EE could increase by up to 15 times resting levels during aerobic exercise and that a bout of exercise of sufficient duration and intensity can alter expenditure post-activity for up to three hours. Alterations up to 8% have been seen in resting metabolic rate in response to aerobic training (Donnelly et al., 2004).

A stronger protective effect of aerobic fitness, compared with physical activity, has been observed (Blair et al., 2001). Individuals with high cardio-respiratory fitness have a metabolic profile including the activity of certain enzymes and alterations in substrate use that are simultaneously related to better weight control and protection against vascular diseases (Telford, 2007). Nevertheless when individuals with similar fitness levels have been compared, health is better in individuals with higher physical activity, thus highlighting the positive health effects of physical activity that are independent of physical fitness (Telford, 2007). Furthermore physical fitness, even in individuals with a high BMI, has been seen to be highly protective against cardiovascular mortality (Fogelholm, 2010). Physical activity improves many of the traditional cardiovascular risk factors such as high-density and low-density lipoprotein cholesterol and triglyceride concentrations, blood pressure and glucose tolerance, however as a stronger association of these risk factors has been observed with obesity in many cross-sectional studies other risk factors must be important in explaining the cardiovascular health effect of physical fitness (Gill and Malkova 2006; Fogelholm 2010).

Physical activity may have favourable effects on lipoprotein distribution, postprandial lipoprotein metabolism, inflammation and endothelial function (Gill and Malkova 2006). Previous work has reported that physical activity may increase the mean size of HDL and LDL particles (Kraus et al., 2002) thus resulting in an atheroprotective effect. Moreover exercise improves postprandial TAG clearance mediated in part by increased lipoprotein-lipase activity and reduced hepatic very low density lipoprotein (VLDL) production (Gill et al., 2002). Exercise improves inflammatory markers by reducing C-reactive protein and the working muscle acts as an endocrine organ by producing interleukin-6 that has anti-inflammatory properties (Petersen and Pedersen 2005). It has also been suggested that exercise up-regulates endothelial nitric oxide synthase gene expression which could further lead to long term structural adaptations and to increased lumen diameter (Fogelholm, 2010). An improvement in insulin sensitivity may also stand as an important explanation for the cardio-protective effects of physical activity and it has been seen to improve sensitivity to a significant degree when sensitivity is impaired, such as in individuals who are obese (Gill et al. 2004). Mechanistically a reduction in intramuscular glycogen levels and increases in the activity of several kinases, leading to the translocation of GLUT-4 to the cell surface results in an improvement in glucose uptake by the muscle cell (Hegarty et al., 2003). Another mechanism leading to increased insulin sensitivity through aerobic fitness is the enhanced fat oxidation which leads to reduced concentration of intramuscular lipids and a decreased activity of protein kinase C. As protein kinase C inhibits insulin receptor tyrosine kinase activity reduced activity of protein kinase C through exercise improves insulin sensitivity (Hegarty et al., 2003).

An increase in EE through adherence to exercise may also result in a compensatory response that may potentially offset the EE of the prescribed exercise once again illustrating the difficulty in successfully implementing weight management programmes in overweight and/ or obese individuals. This means that if one component of the energy equation is altered a second component or multiple components

may compensate which may not necessitate losses of body mass (Colley et al., 2010). For example an absence of body mass loss following the prescription of exercise may be explained by individuals decreasing EE outside of the prescribed exercise sessions thus negating the effect of the exercise on EE it has been suggested (Epstein and Wing 1980). Interestingly when inter-individual differences in adherence are considered for weight management programmes the efficacy of these programmes is seemingly more encouraging. I.e. individuals who adhere to exercise prescription have been observed to lose more body mass and fat mass compared to individuals who had not adhered to advice regarding exercise (Byrne et al., 2006) highlighting the importance of adherence to long term weight management success (Colley et al., 2010). These findings highlight the complexities of prescribing exercise regimes for the purpose of weight loss, particularly in an overweight and/or obese population.

The results of various studies regarding the use of exercise and physical activity on weight loss and the maintenance and improvement of cardiovascular health must be combined in order for a public health message to be constructed. It is clear that the ideal is to be normal weight and physically fit and active however studies have demonstrated that a substantial loss of body weight and the long term maintenance of the weight loss is likely to not be achieved for a large proportion of obese individuals (Fogelholm, 2010). That being said it may be encouraging to be aware that the obesity related health risks can be reduced considerably by increased physical activity and even more if aerobic fitness improves simultaneously (Fogelholm, 2010). The suggestion that physical fitness is apparently more protective than physical activity is another important aspect for consideration although long term maintenance of increased physical activity and high aerobic fitness may again be difficult for initially inactive individuals. The apparent importance of physical fitness over physical activity raises the question of recommended exercise intensity as previously discussed. A review from Fogelholm (2010) suggests that despite the apparent benefits of vigorous exercise a more conservative approach may be preferred for an unhealthy group of people and that the initial threshold for beginning physical activity should be a low amount of moderate intensity as this approach is both feasible and safe for most sedentary individuals (Blair et al., 2001) which can then be increased gradually in those who have already reached the minimal level of health-enhancing physical activity.

Physical exercise has repeatedly been shown to reduce postprandial lipaemia (Petitt and Cureton 2003; Katsanos et al. 2004; Plaisance et al. 2008). Postprandial hyperlipaemia is associated with an increased risk of cardiovascular disease (CVD) through induced symptoms of the metabolic syndrome including insulin resistance, glucose intolerance and hypertension (Petitt et al., 2003). Similarly, high levels of postprandial TAG contribute to atherosclerotic plaque formation and effect endothelial function and are also proposed as a potential CVD risk factor (Zhang et al., 1998). Individuals with or at risk of coronary artery disease have an exaggerated postprandial lipaemia response (Petitt et al., 2003) thus reducing this response is a beneficial outcome worthy of investigation. Individuals with

postprandial lipaemia also tend to have low values of HDL cholesterol and high concentrations of LDL cholesterol again combining as a marker for CVD. The shortening of duration of any postprandial lipaemia may therefore prevent the atherogenic process (Zhang et al., 2007).

Suggested mechanisms for exercise induced improvements include an increase in the activity of the lipoprotein lipase within the capillaries of the exercising muscles, which in turn may accelerate clearance of circulating triacylglycerol (Kolifa et al., 2004) and the improvement of TAG metabolism. The rationale behind such a suggestion stems from the fact that the moderation of postprandial lipaemia is seen within the time frame of enhanced lipoprotein lipase expression and activity following exercise. Another suggestion for such findings includes a decreased secretion of triacylglycerol; however neither suggestion has been strongly supported in experimental studies. Although acute aerobic exercise prior to meal ingestion has been found to attenuate the postprandial lipaemic response, chronic exercise studies in which an acute bout (> 24 hour prior to meal ingestion) were absent have not indicated similarly positive results. In an exhaustive review of the literature up to that point Petitt and Cureton (2003) reported that individuals who perform exercise before the ingestion of a meal exhibit a 0.5 standard deviation reduction in the postprandial TAG response when compared with controls.

A study from Kolifa et al., (2004) indicated that the greatest differences in postprandial plasma total triacylglycerol concentrations compared with and without exercise preceding a meal of moderate fat content (in which significant differences were reported) were found within the first two hours. Postprandial TAG was seen to return to baseline levels in both trials within eight hours post-meal. The lipaemic response has been shown to be positively related to the amount of fat ingested (Dubois et al., 1998) and the energy expended from exercise preceding a meal has been seen to correlate positively with the suppression of postprandial lipaemia (Kolifa et al., 2004).

The effect of exercise timing has been previously addressed by Zhang et al., (1998) who concluded exercise prior to the ingestion of a high fat meal may have beneficial effects on the postprandial TAG response and HDL metabolism, thus, blunting the atherosclerosis process induced by such a meal. This finding was reported when compared with an exercise bout post-meal. Similarly findings from Aldred et al. (1994) were found when compared to a similar study by Hardman and Aldred (1995). Therefore pre-exercise appears to induce beneficial effects on TAG metabolism and postprandial lipaemia measures when compared with post-exercise.

2.16 EXERCISE FOR LOSS OF BODY MASS USING HEART RATE RESERVE

The effect of exercise intensity on weight loss is a current topic of scientific research. In the last two decades a growing number of studies have tested the efficacy of exercise programmes of differing types, weekly frequencies, exercise durations and differing exercise intensities from which position statements and guidelines on the use of physical activity as a therapeutic tool have been informed

(Jakicic et al. 2001; Jakovljevic et al. 2008). Moderate intensity exercise has been described as exercise that ranges from between 40% and 60% of maximal capacity whereas high intensity exercise is that above 65% of maximal capacity although in the majority of studies high intensity exercise refers to that above 80% (Feo, 2013).

One technique of prescribing exercise intensity is through the method of heart rate reserve. Heart rate reserve is described as the difference in age predicted heart rate maximum minus resting heart rate from which a percentage of this value is then calculated often using the Karvonen method (Fletcher et al., 2001). Previous investigations have found significant losses in body weight when exercising at an intensity corresponding to a specific heart rate reserve (HRR) in overweight and obese individuals. A study from Messier and colleagues (2004) highlighted such findings in a group of obese and overweight older adults following a 18-month study in which participants exercised aerobically three times a week for 30 minutes at 50 - 75% HRR and 15 minutes of resistance training with no change enforced on diet throughout the time period. The study reported losses on average of 3.7% body mass equating to 3.46 kg in individuals (Messier et al., 2004). Interestingly a group in which the same exercise regime was implemented in conjunction with a dietary intervention reported greater losses of 5.7% body mass equating to 5.2 kg on average further highlighting the aforementioned synergistic effect of diet and exercise. A similar exercise protocol in which middle aged and older men exercised for 30 minutes three times a week by walking, jogging or cycling initially between 50-75 % HRR for six months before progressing to 80 – 85% HRR reported significant losses in body fat percentage, waist circumference and waist to hip ratio (Pratley et al., 2000).

2.17 EXERCISE AND EXPOSURE TO ENVIRONMENTAL HYPOXIA ALTERS BODY MASS, BODY COMPOSITION AND METABOLIC FACTORS

OVERVIEW

Previously within this review of literature the effect of hypoxic exposure on weight loss has been explored. Studies in which physical activity has been considered whilst resident at high altitude were described in this previous section. The current section however focuses on investigations in which the combined stimuli of structured and planned exercise programmes and exposure to environmental hypoxia are combined in order for the effect of the two stimuli to be reviewed. The technical developments in the production of artificial climates including both normobaric and hypobaric hypoxic chambers at relatively low energy costs has allowed for an increase in controlled experimental trials with stable experimental conditions in overweight and obese individuals. The combination of exercise and exposure to reduced FiO_2 or reduced partial pressures has a number of potential variations worthy of investigation including losses of body mass, loss of body fat, changes in substrate utilisation and the regulation of pathways that are crucial to glucose and lipid metabolism (Wiesner et al., 2010) coupled with improvements in cardiovascular health including insulin sensitivity and blood pressure.

MAIN FINDINGS AND MECHANISTIC EXPLANATIONS

Regular physical activity improves cardiovascular and metabolic risk factors (Donnelly et al., 2009). The question of how training may be optimised such that overweight and obese populations gain maximal metabolic and cardiovascular benefit while minimising injury risk is one of importance. The synergistic effect of exposure to environmental hypoxia and physical activity on both cardiovascular and metabolic risk factors has recently been highlighted (Netzer et al., 2008; Haufe et al., 2010; Wiesner et al., 2009). Work from Netzer et al., (2007) provided the first study, to the author's knowledge, to incorporate sham hypoxia and exercise in a controlled investigation. Under moderate hypoxia (FiO_2 : 0.15) obese subjects exercised at a heart rate corresponding to 60% of their maximum O_2 consumption three times a week for eight weeks. This led to significantly more weight loss compared with that of the control group, who also exercised at a heart rate corresponding to 60% of their maximum O_2 three times a week but in a normoxic environment. Participants in the hypoxic group lost an average of 1.14 kg versus an average of 0.03 kg in the control and also exhibited non-statistically significant tendencies to decrease cholesterol, TAG and low-density lipoproteins (LDL) compared with that found in the control group. Interestingly participants in the hypoxic group exercised at a lower absolute workload due to the hypoxic effect on heart rate which may have beneficial effects when working with overweight populations.

In a similar study from Wiesner et al., (2009) a single blind study in obese patients was conducted to test the hypothesis that training under hypoxia results in similar or greater changes in body composition and metabolic risk factors compared with exercise in normoxia (Figure 2.17). This hypothesis was formed in light of previous findings reporting an increase in hypoxia-inducible factor1 (Ameln et al., 2005), the up-regulation of Leptin (Yingzhong et al., 2006) and the increase in peroxisome proliferator-activated receptor- γ co-activator-1 α mRNA expression (Zoll et al., 2006) from training in hypoxia. Furthermore in a pilot study carried out by the same group (Haufe et al., 2008) lean healthy men exhibited similar or even better responses in terms of cardiovascular and metabolic risk factors following a four week period in which they trained in normobaric hypoxia compared to normoxic training at a heart rate measured at 3 mmol.L⁻¹ of lactate. A similar four week training programme, during which subjects completed 60 minutes of exercise, three times a week corresponding to 65% of $\dot{V}\text{O}_2$ max in hypoxia or in normoxic conditions, was employed. Individuals in both groups remained weight stable throughout the investigation although significant improvements in body fat content were reported in the hypoxic training group so that a reduced body fat percentage and a concomitant increase in fat-free mass was observed (Figure 2.18). This greater reduction in body fat in the hypoxia group is speculated to have been due to improved lipid oxidation. Improvements in time to exhaustion, $\dot{V}\text{O}_2$ max and the Homeostasis Model Assessment (HOMA) index were also seen to be similar in both groups however reductions in the respiratory quotient and lactate response at the individual anaerobic threshold were seen only in the hypoxic group as was a decrease in diastolic blood pressure. Due to the hypoxia induced increase in HR these results were reported with the

hypoxic group exercising at a significantly lower workload (17.5%) compared to that of those in the control. This again highlights the potential for the use of such methods in an applied setting where exercise capacities are lower amongst the obese and the risk for orthopaedic injury may be reduced if exercise intensity is reduced.

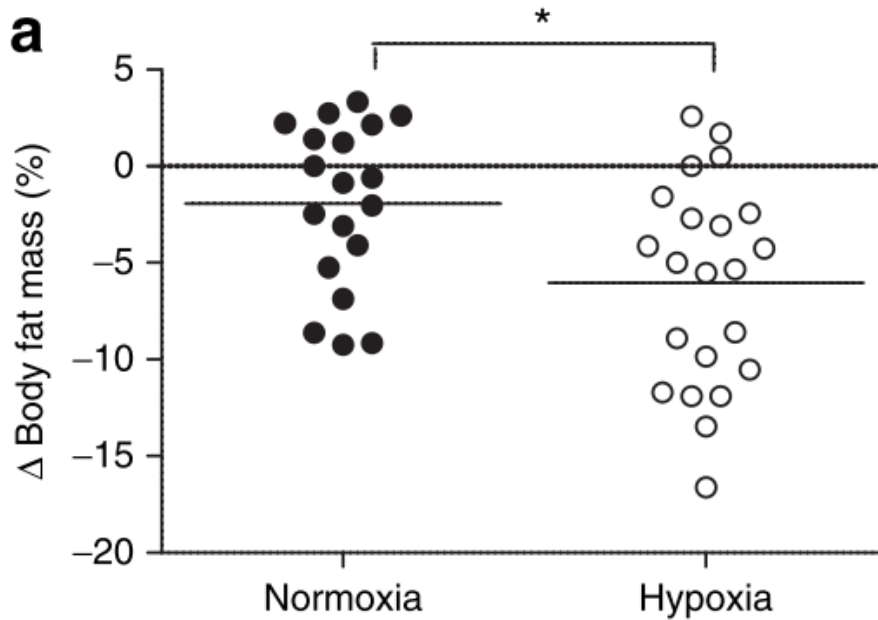


FIGURE 2.17: Change in body fat mass % in normoxia and hypoxia groups following the four week exercise training programme (Wiesner et al., 2010).

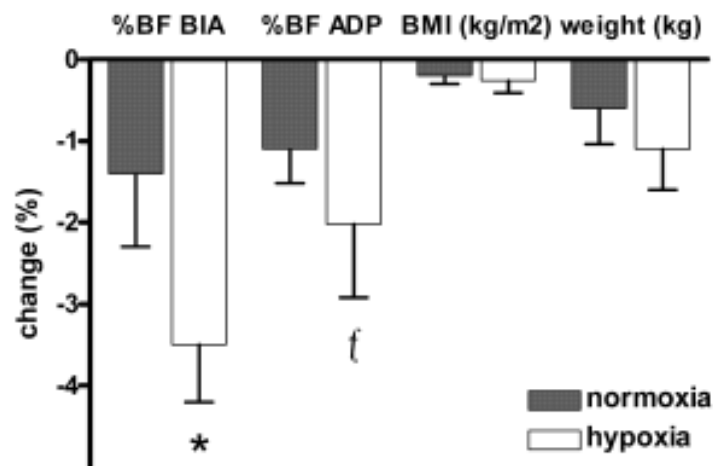


FIGURE 2.18: Percentage Change in body fat, body mass index, and body mass between normoxia and hypoxia groups (Haufe et al., 2008).

Notes: Data are presented as mean \pm SEM. * $p < 0.05$, $\dagger p = 0.073$ Bioelectrical impedance (BIA), air displacement plethysmographie (ADP), body mass index (BMI).

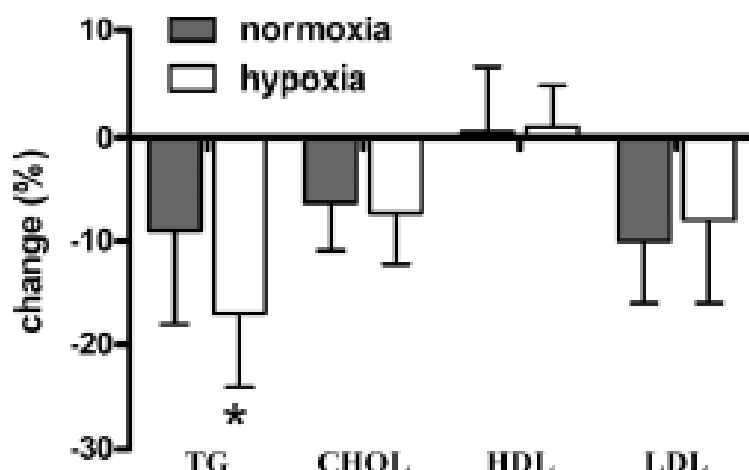


FIGURE 2.19: Changes in triglycerides (TG), cholesterol (CHOL), high density lipoprotein (HDL) and low density lipoprotein (LDL) between normoxia and hypoxia groups (Haufe et al., 2008).

Notes: Data are presented as mean \pm SEM. * $p < 0.05$

In the aforementioned pilot study (Haufe et al., 2008) the combination of exercise and hypoxia had a seemingly greater effect on triglyceride concentration than each stimulus alone in lean healthy men Figure 2.19. The beneficial effect of endurance training on triglyceride levels has been attributed to increased post-exercise lipid oxidation (Kuo et al., 2005) which may also be seen through hypoxic exposure (Zoll et al., 2006). No effect on triglyceride concentration however was observed in obese participants (Wiesner et al., 2010).

In an attempt to control for the “common flaw” of a lack of dietary control in previous work the effect of hypoxic training coupled with a low calorie diet has been examined in young adults who are obese (Kong et al., 2013). The use of a residential camp, which has been shown previously to be successful in inducing weight loss in obese children and adolescents (Kelly and Kirschenbaum 2011), was employed for four weeks coupled with the hypo-caloric control in 22 individuals between the ages of 17 to 25 years. During the camp participants were assigned to hypoxic training or normoxic training and dietary intake was restricted to the energy intake of each individual’s desirable weight. On average the daily caloric intake deficit was 637 ± 333 kcal in the hypoxic group and 691 ± 330 kcal in the normoxic group and the physical activity conducted was expected to burn 4,000 kcal per week over the course of 11 sessions which included running, stepping, cycling and dumbbell exercises usually conducted at 60-70% of maximum heart rate. The hypoxic training group completed sessions in an environment of FiO_2 : 0.16 (2,000m) which gradually reduced to FiO_2 : 0.14 (3,000m). Losses of weight occurred in both hypoxic and normoxic groups following the four weeks with the decrement significantly greater in the hypoxic group (-6.9 kg vs -4.3 kg). Reductions in BMI, fat mass and waist to hip ratio were also significantly greater following hypoxic training compared to normoxic training. The

study suggests that previous studies in which the effect of hypoxic training on losses of body mass may have underestimated the effect of such stimuli through the lack of dietary control and extra physical activity undertaken whilst at altitude. Mechanistic suggestions regarding the effect of additional losses of weight reported in conjunction with hypoxic training include increased EE, appetite suppression and responses of neuroendocrine factors relevant to energy balance (Kong et al., 2013). Furthermore a decrease in systolic and mean blood pressure was seen following hypoxic training only which may be explained by the reductions in fat mass it is suggested or possible through the hypoxic induced increase of arteriole diameter resulting in peripheral vasodilation and decreased peripheral resistance in the systemic circulation (Urdampilleta et al., 2011). Moreover it was observed in the study that the hypoxic group completed a greater distance during their aerobic training sessions suggesting an improved quality of training. This is perhaps due to improved exercise performance gained through the intermittent hypoxia thus resulting in an additive physiological effect which may in turn explain some of the benefits recorded from hypoxic training compared to training in normoxia.

The development of insulin resistance is known to be associated with regional fat mass including visceral fat area and intramyocellular lipid content; a recent study from Morishima et al., (2014) investigated the effect of hypoxic training on these measures in twenty healthy sedentary males. A four week training programme was conducted, during which participants exercised three times per week in either hypoxic or normoxic conditions consisting of 60 minutes of cycling at 55% of maximal oxygen uptake. As in previous studies, the hypoxic group exercised at a significantly lower absolute workload than those in the normoxic group although no differences in heart rate were observed. The study concluded that hypoxic training showed a greater improvement in postprandial glucose tolerance compared with normoxic training although no further reductions in measures of whole body fat mass, abdominal or intramyocellular fat content were observed. The detailed mechanism underlying the significant improvement in glucose tolerance without a loss of whole body fat mass from hypoxic training is not fully understood. It is suggested however the enhancement of glucose uptake by skeletal muscle may be involved (Morishima et al., 2014a).

In a study from Bailey et al., (2000) the synergistic effects of cycling exercise and intermittent normobaric hypoxia on metabolic and cardiovascular risk factors was investigated in healthy males. More specifically the aim of the investigation was to examine any additional effects of hypoxia on the normal physiological adaptations to physical exercise. This study was partly based on previous suggestions that an inverse relationship between coronary mortality and altitude residence exists (Voors and Johnson 1979) thus suggesting a role for hypobaric hypoxia as a modulator of blood lipid metabolism. Furthermore previous reductions in total cholesterol, LDL cholesterol, triglycerides (Férézou et al., 1988) and systemic blood pressure (Brinchmann-Hansen and Myhre 1989) during an acute altitude stay informed the study's research question. The study observed, in contrast to some similar studies previously presented, that both relative and absolute training intensities in the hypoxic and normoxic groups were identical. The study was also able to control for confounding factors

including cold, malnutrition and increases in physical activity that are often present at high altitude by administering hypoxic air to the participants in a double blind design in a laboratory environment. An increase in lean body mass was observed in the hypoxic training group only so that individuals increased their lean mass by 1.4 ± 1.5 kg with no changes in dietary composition or nutrient intake. A clinically significant decrease in maximal systolic blood pressure and rate pressure product was observed following hypoxic training alone in the study from Bailey. A reduction in rate pressure product is representative of a significant decrease in cardiac workload and is considered a positive adaptation with the potential of cardio-protective implications (Bailey et al., 2000). Although the exact mechanisms for the reductions in blood pressure are not entirely known a physiological adaptation to intermittent hypoxaemia may upregulate vascular reactivity. These findings may have important application to individuals who are at greater risk from cardiovascular disease as it is seen that those individuals respond more favourably to physical exercise training (Lokey and Tran 1989). Although healthy males were used for the study it is argued that improvements in cardiovascular disease risk following an increase in physical activity can occur (Ketelhut et al., 1996) thus suggesting that an upper limit of dose-response between physical activity and cardiovascular disease risk may be non-definable.

In summary previous studies have investigated the clinical benefits of hypoxic training on cardio-metabolic risk factors including body weight, body composition, blood glucose, blood pressure and blood lipid levels. From this previous work it can be observed that overall the use of moderate levels (≥ 1500 m) of hypoxic stay has resulted in improvements in body weight (Lippl et al., 2010) and that in order for these losses in body weight to occur; continuous exposure of at least one week at a minimum 1,700m of altitude is required (Greie et al., 2006; Lippl et al., 2010). Intermittent hypoxic training used in conjunction with moderate exercise three times a week has been sufficient to significantly increase lean body mass (Bailey et al., 2000). Exercise intensities that have been successful in bringing about positive outcomes in body composition and bodyweight have ranged from 55 to 85% heart rate max and overall the use of moderate levels ($\geq 1,500$ m) of hypoxic exposure as a supplement to exercise training have resulted in improvements in body composition and body weight (Wee and Climstein 2013). Extreme altitudes however have shown to have an adverse effect on fat free mass due to excessive hypoxic stress (Wagner, 2010).

The effects of acute hypoxic training on blood pressure often results in no changes in systolic and diastolic values (Snyder et al. 2009; Wee and Climstein 2013). Chronic exposure, lasting longer than three weeks has however resulted in mixed findings with decreases in systolic and diastolic values (Greie et al., 2006) increases (Mori et al., 1999; Siqués et al., 2009) and no changes (Wiesner et al., 2010) all having been reported. Improvements in blood pressure values from moderate levels of altitude (1,700 – 2740m) in individuals with hypertension have been observed however with improvements of 8 – 13 mmHg for diastolic values (Greie et al., 2006; Lippl et al., 2010) and up to 26 mmHG for systolic values (Auer et al., 2004). A significant reduction in total cholesterol has also

commonly been reported in investigations in which unhealthy participants were examined following hypoxic training (Bailey et al., 2001; Greie et al., 2006; Wagner, 2010).

In light of the current literature suggested mechanisms for the improvement of body composition and the reduction of body weight following the combination of hypoxic exposure and exercise training include an increase in metabolic rate which can result in an altered substrate utilisation and improved mitochondrial oxidative capacity via signalling pathways that stimulate GLUT-4 transport (Roels et al., 2014). Training in hypoxic conditions may also allow for a higher relative intensity to be achieved which would reduce the mechanical strain of higher workloads while gaining similar benefits (Wiesner et al., 2010). A suppression of appetite may also be induced through hypoxia, as previously discussed within this review of literature, thus resulting in a loss of body mass and improvement in body composition. Furthermore a hypoxic induced increase in adrenaline levels may result in increased glycolysis (Kon et al., 2010). Although these studies can provide only suggestions as to the exact mechanisms behind the findings there is a greater wealth of evidence reporting that even mild hypoxia in both normobaric and hypobaric when combined with exercise can have promising benefits on some metabolic risk factors including weight loss, changes in body composition, blood glucose and blood pressure.

SUMMARY

The beneficial effects of exercise for weight loss have been investigated. This effect acts primarily through increased EE and the modulation of appetite (Donnelly et al., 2009). Previous reports of an increased metabolic rate in humans exposed to an environment of hypoxia have provided an alternative method to induce a negative energy balance (Kayser et al., 2013). The combination of exercise and exposure to hypoxia has therefore been suggested as a viable method to induce a meaningful increase in EE, a subsequent reduction in body mass and an improvement in cardio-metabolic health in overweight and obese individuals.

The potential for larger hypoxemia when physical activity is performed at reduced oxygen availability has been suggested as a possible explanation for recent positive findings. Homeostatic perturbations induced upon exposure to hypoxia are similar to changes induced by physical activity. During altitude sojourns said findings have included reductions in body mass, body fat, high density lipoprotein concentration and both systolic and diastolic blood pressure although debate remains regarding the specific effect of hypoxia per se independent of physical activity due to lack of control groups. In chamber studies however greater weight loss, improvements in body composition, and improvement in several metabolic risk factors including body fat content, fasting triglycerides and fasting insulin levels have been reported in overweight individuals completing low to moderate intensity training programmes in hypoxia compared to normoxia (Haufe et al., 2008; Netzer et al, 2008; Wiesner et al., 2010). Other relevant findings include a synergistic effect of exercise and hypoxia in reducing appetite explained by decreases in plasma acylated Ghrelin concentrations (Bailey et al., 2015) and the

observation that improvements in metabolic parameters have been observed through a lower training workload and thus a reduced mechanical load (Netzer et al, 2008; Wiesner et al., 2010).

Taken together these findings support the use of exercise coupled with hypoxia as a useful method for improvements in body mass, composition and related metabolic risk factors in overweight individuals and suggest that these effects are greater when compared to exercise alone. Further research however is required in order to elucidate the underpinning mechanistic reasons for the findings. Future work should also focus on longer term interventions of differing severities of hypoxia, and of differing modes and intensities of exercise. Moreover the effect of exposure to hypoxia at rest preceding an exercise bout may be investigated in order to assess any lasting effects of hypoxia induced perturbation during exercise.

2.18 AIM OF THESIS

To investigate the metabolic response of humans to acute and intermediate term hypoxia. Specifically the effect of hypoxia on factors that potential contribute to alterations in body mass and composition.

The acute effect of severe hypoxia on resting metabolic measures in healthy participants will initially be quantified. Namely measures of resting metabolic rate, substrate oxidation, and circulating blood lipid responses. The effect of acute hypoxia and moderate-intensity exercise on the same measures will then be examined.

The immediate and lasting effects of a more prolonged exposure to reduced oxygen availability will then be investigated primarily assessing the response of body mass, composition, substrate use and blood lipid values. The effect of moderate altitude stay on sensations of taste will also be evaluated in an attempt to highlight contributory effects of altered appetite at altitude. Finally a study investigating the metabolic response to a high fat meal following an intermediate length stay at altitude will be conducted.

The acute and prolonged effect of hypoxia on circulating levels of Meteorin-like will be quantified throughout this thesis.

2.18.1 RESEARCH QUESTIONS ARISING FROM THE LITERATURE REVIEW

The literature review forming this thesis has identified a number of areas for investigation still outstanding. It is understood upon exposure to conditions of reduced oxygen availability numerous physiological responses occur in humans. Such alterations are dependent upon, amongst other factors, the severity and length of exposure, the method and time frame of ascent and the nature of the hypoxia i.e. normobaric or hypobaric. The current prevalence and the predicted increase in obesity and its associated metabolic conditions highlights the importance of reducing the number of cases in the UK and Worldwide as a central issue of clinical research. It has been suggested that exposure to altitude/hypoxia may provide therapeutic effects.

Commonly observed upon prolonged exposure to hypoxia is a reduction in body mass and a subsequent alteration in body composition. Whilst the mechanisms associated with said alterations are now better researched there is still much to be understood with regard to contributing factors. Currently suggested mechanistic explanations with regard to the effect of hypoxia per se include changes in metabolic rate, a shift in the oxidation of carbohydrate and fat sources, the malabsorption of nutrients and a reduction in appetite and subsequent eating behaviour. Further progression is required however with regards to the use of short term exposure both independently and in conjunction with exercise on physiological responses that may aid the development of hypoxic exposure as a tool for inducing beneficial metabolic effects. Specifically the quantification of changes in substrate use and metabolic rate during a short bout of hypoxia will be beneficial. Furthermore a greater understanding of the interplay between moderate intensity exercise in conjunction with a

short term exposure may supply information that is practically important for the development of a beneficial therapeutic programme.

Fat “browning” has been previously observed upon exposure to cold conditions in rodents and is a potential method for increasing EE. This review of literature highlights the potential of the use of environmental extremes as a method to induce greater EE through the “browning” of beige adipocytes. Greater research is required and warranted with regards to the effect of hypoxia on such responses and with regards to fat “browning” in humans however. Specifically the use of both acute and moderate term exposure on Metrnl concentration will improve our understanding. Meteorin-like is affected by stimuli including physical activity and cold exposure with expression increased following acute cold exposure (six hours at 4°C and 24 hours at 4°C) but not chronic exposure (two weeks at 4°C) (Rao et al., 2014). An increase in sympathetic activation is observed upon exposure to both high altitude and extreme cold potentially explaining the observed increases in metabolic rate upon exposure to altitude (Butterfield et al. 1992; Mawson et al., 2000) A hypothesised increase in Meteorin-like upon exposure to hypoxia/altitude should be examined and may provide a mechanism for altitude induced reduction in fat mass. Increased sympathetic nervous system activity upon exposure to hypoxia/altitude may drive these increases.

2.18.2 PROPOSED RESEARCH STUDIES AND HYPOTHESES

The following research questions and associated hypotheses are proposed for this thesis

EXAMINATION OF THE REPRODUCIBILITY AND VALIDITY OF THE METAMAX3X IN NORMOBARIC HYPOXIA AT REST

Quantify the accuracy of repeated ventilatory measures at rest in an environment of normobaric hypoxia for 45 minutes. Determine the validity of ventilatory measures at rest in an environment of normobaric hypoxia for 45 minutes against the Gold-standard Douglas bag method.

- It is hypothesised that the MetaMax 3X will display accuracy in the measure of ventilatory parameters at rest on repeated occasions in normobaric hypoxia. Secondly it is hypothesised that the MetaMax 3X will display good agreement with the gold standard Douglas bag method of collection.

THE METABOLIC RESPONSES AT REST TO ACUTE, NORMOBARIC HYPOXIA (FI_O₂: 0.12)

Quantify the response of resting metabolic rate, cardiovascular stress, substrate oxidation, plasma free fatty acids (FFA) and triglycerides (TAG) during exposure to an acute, normobaric bout of hypoxia (FI_O₂:0.12). Such quantification may provide important information regarding the potential use of acute exposure in isolation or in conjunction with exercise as a therapeutic tool for obesity and related metabolic disorders.

- It is hypothesised that an acute exposure to severe normobaric hypoxia will induce increases in resting metabolic rate and thus increases in the oxidation of fat and carbohydrate derived sources. It is also hypothesised that an increase in circulating levels of plasma FFA will occur.

THE METABOLIC RESPONSES TO ACUTE, NORMOBARIC HYPOXIA (FI_O₂:0.12), AND MODERATE INTENSITY EXERCISE FOLLOWING A HIGH FAT MEAL

Quantify the independent effects of normobaric hypoxia (FI_O₂:0.12), moderate intensity exercise (60% heart rate reserve) and a high fat meal on resting metabolic rate, substrate oxidation and plasma concentrations of Meteorin-like.

Quantify the combined effects of exposure to hypoxia at rest followed by moderate intensity exercise in normoxia on resting metabolic rate, substrate oxidation and plasma concentrations of Meteorin-like.

Quantify the combined effects of a high fat meal followed by exposure to hypoxia at rest followed by moderate intensity exercise in normoxia on resting metabolic rate, substrate oxidation and plasma concentrations of Meteorin-like.

- It is primarily hypothesised that acute hypoxic exposure will increase EE during rest. Secondly It is hypothesised an increase in fat through a high fat meal in combination with hypoxia will

increase fat use during subsequent exercise. Finally it is hypothesised that the combination of acute hypoxia and moderate intensity exercise may act together to promote the release of MetrnI and increase fat use as a fuel.

THE ACUTE AND LASTING METABOLIC RESPONSES TO AN INTERMEDIATE TERM STAY AT MODERATE HYPOBARIC HYPOXIA (3,400M)

Determine the effect of an 18 day stay at a moderate altitude of 3,400m on measures of body mass and body composition throughout the sojourn and during a follow up period upon return to sea-level. The quantification of resting metabolic rate, substrate oxidation, MetrnI and taste thresholds will identify contributing factors to such changes in body mass and composition and may improve our understanding of mechanisms behind altered eating habits, metabolic changes and the reasons for induced cachexia.

- It is primarily hypothesised that an 18 day stay at moderate altitude will induce lasting reductions in body mass, increase RMR and acute reductions in appetite and taste sensation. Secondly it is hypothesised that MetrnI concentrations will increase in response to the altitude stay.

THE METABOLIC RESPONSES TO A HIGH FAT MEAL FOLLOWING AN INTERMEDIATE TERM STAY AT HYPOBARIC HYPOXIA (3,400M)

Quantify the response to the ingestion of a high fat meal following an 18 day stay at moderate hypobaric hypoxia in order to elucidate the lasting effects of sustained hypoxia on blood lipid parameters following a known lipid intake.

- It is hypothesised that an 18 day stay at moderate altitude will induce beneficial effects on the response to the ingestion of a known lipid intake.

3. GENERAL METHODS

This chapter describes the materials and methods used in all experimental chapters of the thesis. All laboratory based methods were conducted in the British Association of Sport and Exercise Sciences (BASES) accredited Welkin Laboratories at the University of Brighton. Additional or modified materials and methods used in experimental chapters will be described within the specific methods section.

3.1 ETHICS, HEALTH AND SAFETY

All investigations reported in the transfer document were approved by the University of Brighton Ethics and Governance committee and conducted in accordance with the guidelines of the revised declaration of Helsinki 2013. All experiments were carried out in line with the University of Brighton standard operating procedure and risk assessment laboratory guidelines.

3.2 EXPERIMENTERS

During each data collection session at least two experimenters were present throughout, at least one of whom was qualified at first aid and to use an automated external defibrillator. For experimental chapters where the use of the hypoxic chamber is described, two experimenters were separated so that one was located within the hypoxic chamber attending to the participant, and one experimenter was located external to the chamber to ensure health and safety of both individuals exposed to hypoxia.

3.3 EQUIPMENT CLEANING AND CONTROL OF SUBSTANCES HAZARDOUS TO HEALTH

To avoid contamination all apparatus was cleaned before and after use. Metabolic gas collection equipment such as facemasks, falconia tubing, mouthpieces and nose clips were soaked in Virkon disinfectant (1% Antec Int. Suffolk, UK) for a minimum of 10 minutes, followed by a thorough rinse in cold water and drying prior to use as per manufacturer guidelines. Heart rate monitor straps were soaked for 10 minutes in 1% Virkon disinfectant, rinsed and dried following use.

3.4 WASTE DISPOSAL

Biological material and waste were handled and disposed of in line with relevant guidelines. Control of Substances Hazardous to Health (COSHH) sheets were completed for every power or solution used within the study. Risk assessments were also completed for use of all laboratories, hypoxic facilities, exercise and invasive techniques such as cannulation.

All other non-reusable waste were disposed of by immediate placement in marked biohazard waste containers and incinerated. Sharps, such as venepuncture needles, were also disposed of in marked sharps containers and subsequently incinerated. Electrical equipment contacting the body such as heart rate monitors, were cleaned using warm water and soap, followed by alcohol cleaning wipe.

3.5 PARTICIPANTS

3.5.1 MEDICAL CRITERIA AND RECRUITMENT

Healthy individuals (males and females) with a range of BMIs were recruited for each study (20-28 kg/m²). Participation in each experiment was conditional upon the completion of a medical questionnaire (see appendix part 2) - with no contraindications for participation. Participants were excluded from participation if they had been verified, or documented as having any blood carried infections (Hepatitis, HIV), were diabetic, or presented with a known history of haematological, cardiac, respiratory, or renal disease.

Experimental trials were started only after participants involved provided written informed consent (see appendix part 1). For each individual experimental study, participants were provided with a participant information sheet detailing the study design, participant requirements and risks and benefits, written using lay terms. Participants were also invited to ask questions regarding the study, before they consented to undertaking the research. Participants were informed that they could withdraw from the study at any time without providing justification or explanation, or without incurring any penalty.

3.6 PRE-TRIAL DIET AND EXERCISE STANDARDISATION

Participants were instructed to maintain normal hydration and to abstain from participating in vigorous physical activity or exhaustive exercise and alcohol consumption 48 hours prior too, and throughout the duration of each experiment. Experimental trials were conducted at the same time of day for all participants to avoid an effect of circadian variation on physiological variables (Consoli et al., 1981). Participants were instructed to complete a 12 hour overnight fast prior to testing, having consumed their last meal before 20:00 on the preceding evening and to maintain their normal diet throughout the time period of testing. Participants were non-smokers (Agarwal et al., 2014) and had not spent any time above 2000m in the preceding 2 months and had not flown in the previous 3 months with the exception of studies 4 and 5 (Chapters 7 and 8). Participants had been absent from repeated external heat exposure for the previous three months. Participants were excluded from taking part if they were taking any medication or dietary supplements, had taken part in other laboratory experiments in the three months preceding, had experienced anaphylactic shock symptoms to needles, probes or other medical equipment that might preclude them from having a cannula inserted. The confounding variables of caffeine (Lu et al., 2008), and alcohol (Taylor et al., 2010) were controlled for at sea-level and altitude. Specifically participants were instructed to avoid alcohol for the 48 hour period prior to testing and to avoid caffeine for a period of 12 hours prior to testing. Female participants were tested throughout the menstrual cycle thus the use of contraceptive medication is likely. Basal and resting metabolic rate is effected by phases of the menstrual cycle (Solomon et al., 1982). Specifically BMR decreases at menstruation and is observed to be at its most reduced approximately one week prior to ovulation prior to increasing until the beginning of the next

menstrual period (Solomon et al., 1982). Such variation in metabolic rate is considered within the analysis and interpretation of results in studies where females were recruited.

3.7 CRITERIA FOR TERMINATION OF EXPERIMENTS

Experiments were stopped if any of the following criteria were met:

- The participant asked to stop the test. Participants were not required to give any reason for this;
- The experimenter felt it appropriate to stop the test whether it be for equipment issues, or the participant displaying signs of discomfort or illness, including, but not limited to chest pain, dyspnea, nausea, vomiting, generic pain/discomfort, faintness or dizziness;
- If both a LLQ score of 6 or above and a SpO_2 of less than 75% were recorded.

The SpO_2 safety limit is set by the University of Brighton Research Ethics Committee.

3.8 HYDRATION ASSESSMENT

To ensure equal and adequate hydration between trials the assessment of hydration status was performed. When the following criteria were achieved, adequate hydration to perform the trial was assumed; an osmolality value of $\leq 700 \text{ mOsm.kg}^{-1}$, a urine specific gravity value of ≤ 1.020 , (Sawka et al., 2007). These experimental controls were not violated for any participant for any of the experimental procedures.

3.9 URINE OSMOLALITY

Urine osmolality was measured using a handheld micro osmometer (Advanced Instruments Inc., Massachusetts, USA). The micro osmometer was calibrated prior to every sample using distilled water ($0 \text{ mOsm.kgH}_2\text{O}^{-1}$). Approximately 1mL of urine was placed into the osmometer lens, whereby the sample was measured using water freezing point depression.

3.10 URINE SPECIFIC GRAVITY

Urine specific gravity (USG) was assessed using a visual handheld refractometer (Index Instruments Ltd., Cambridge, UK). The refractometer was calibrated prior to every sample using distilled water (USG 1.000). Approximately 2 mL of urine was placed onto a glass lens of the refractometer. The refractometer was then held up to the light, while the experimenter looked through the eye lens and recorded the values from the scale within.

3.11 ANTHROPOMETRIC ASSESSMENT

Height was measured using a fixed stadiometer (SECA). Participants were required to stand vertically in the anatomical position facing away from the stadiometer scale into the laboratory. The stadiometer arm was lowered until it rested horizontally on the most superior aspect of the head. The scale was then read to the nearest 0.5 cm.

3.11.1 BODY MASS

Nude body mass (NBM) was recorded to the nearest 10g using Adam GFK 150 weighing scales (Adam Equipment Inc., Connecticut, USA). The scales were calibrated prior to use, using a 20 kg weight. Participants were required to stand nude on the plate until the digital display stabilised. This procedure was carried out by participants in a private room, participants self-reported their body mass.

3.11.2 BODY FAT PERCENTAGE

Assessment of body fat was measured using Tanita bioelectrical impedance (BIA) (Tanita TBF-300A Body Composition Analyzer/Scale, IL, USA) using the wrist and foot electrode connecting method. Participants were instructed to lie at rest for a period of 30 minutes prior to the measure being taken. Participants had performed a 12 hour fast prior to the measurement and had not performed exercise, or consumed alcohol or caffeine in the 12 hours prior to body fat assessment.

3.12 ENVIRONMENTAL CONDITIONS

3.12.1 AMBIENT LABORATORY TEMPERATURE CONTROL

Seated rest for the measurement of resting values and preliminary testing were performed in ambient laboratory conditions at $20 \pm 1^\circ\text{C}$ and $40 \pm 5\%$ using industrial air conditioning (Daikin, UK). Barometric pressure was determined from a portable barometer (Weather station, Oregon Scientific, Oregon, USA).

3.12.2 CONTROLLING THE HYPOXIC ENVIRONMENT

A large, purpose built hypoxic chamber (The Altitude Centre, UK) using nitrogen enriched air was used to simulate altitude through creating the hypoxic environment. The chamber using computer controlled generators was set at a given FiO_2 which was then monitored and controlled through computer software (The Altitude Centre, UK) and confirmed by three O_2 sensors affixed around the internal and external walls of the chamber within the laboratory. Thermal control of the hypoxic chamber was facilitated by a large internal air conditioning unit set to 20°C . Manual recording of the FiO_2 was performed every 5 minutes to accurately describe the environment experienced by participants.

3.13 CARDIOPULMONARY MEASURES

3.13.1 DOUGLAS BAG METABOLIC GAS ANALYSIS

Gas analysis using both Douglas bag and online metabolic analyser methods were employed. Expired gas was collected in Douglas bags (Type 343, Georg Fischer, Switzerland). During data collection the participants were instructed to remain quiet in a supine position but to remain awake. All Douglas bags were evacuated immediately prior to data collection. During the Douglas Bag trial expired gas was collected into Douglas bags over approximately 60 seconds every 5 minutes. A 60 second data collection has previously been shown to provide similar values with a mixed chamber gas system highlighting this collection period as suitable for measures of $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER (Nieman et al., 2013). After the experimenter had washed their hands, mouthpieces, valves and Falconia tubing were assembled, with the nose-clip ready to use.

Subjects were habituated to breathing through the mouthpiece before each collection and were accustomed with the apparatus for 30 seconds before collection commenced. Samples were collected over a complete number of respiratory cycles from end expiration to end expiration. The exact collection time was recorded. The gas analyser (Servomex 1400, Servomex Group Ltd., England) was calibrated prior to each data collection session. During analysis of each Douglas bag data recorded included O_2 and CO_2 percentage. Inspired O_2 and CO_2 fractions were monitored constantly using a sample tube connected directly to the infra-red capnograph gas analyser. Analysis for O_2 and CO_2 was timed for exactly 60 seconds (flow rate = $0.5 \text{ L}\cdot\text{min}^{-1}$), with recording of gas percentages occurring upon stabilisation after approximately 45 seconds. Volume and temperature of expired air were measured using the dry gas meter with the gas volume meter zeroed prior to each evacuation. Gas volume was evacuated at a rate of $1 \text{ L}\cdot\text{min}^{-1}$. Values for oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation \dot{V}_E , RER were calculated using the Haldane equation. The participants were instructed to remain quiet in a supine position during data collection but to remain awake.

Indirect calorimetry is the traditional method for estimating metabolic EE (Weir, 1949). This approach requires the measurement of the rate of oxygen (O_2) consumption and carbon dioxide (CO_2) production. Using the energy equivalent for the full oxidation of carbohydrates (21.13 kJ per L of O_2) and fats (19.63 kJ per L of O_2 consumer), metabolic EE can be subsequently calculated (Kenny and Jay 2013).

Equation 3.1: Calculation for metabolic energy expenditure

$$M = [\dot{V}O_2 (((RER - 0.7) / 0.3) e_c + ((1.0 - RER) / 0.3) e_f)] / 60$$

Where: $\dot{V}O_2$ is measure in $L \cdot \text{min}^{-1}$; e_c is the caloric equivalent per litre of oxygen for the oxidation of carbohydrates (21130 J), e_f is the caloric equivalent per litre of oxygen for the oxidation of fat (19630J) and RER is the respiratory exchange ratio.

3.13.2 ONLINE GAS ANALYSIS

The MetaMax3X was calibrated according to normal operational procedures. Before calibration and testing the MetaMax3X was switched-on for at least 45 minutes. Calibration involved a three-point process in which atmospheric pressure, volume and gas were all calibrated respectively. Atmospheric pressure calibration consisted of the use of a digital barometer from which actual values in mbar are transferred to the device following conversion to offset values. Volume calibration required simulated inspiration and expiration via manual syringe for five accepted strokes of a Hans Rudolph 3-litre syringe at eliciting a flow rate of 60 litres.min⁻¹. Gas calibration is conducted using ambient air (assumed to be 20.93% O₂ and 0.03% CO₂) and requires two known O₂ and two known CO₂ gas concentrations known as Gas 1 and Gas 2, appropriate for use in hypoxic conditions, to be sampled until stabilisation of sensors had been achieved.

Gas 1 was required to represent the inspired fraction and Gas 2 the expired fraction. For ambient trials, Gas 1 was taken directly from the ambient environment ($F_{iO_2} = 0.2093$ $F_{iCO_2} = 0.0005$), Gas 2 was drawn from a contained gas cylinder ($F_{iO_2} = 0.17$ $F_{iCO_2} = 0.05$) into a clamp sealed collection bag which was immediately affixed to the sample line. For hypoxic trials Gas 1 was taken directly from the hypoxic environment ($F_{iO_2} = 0.12$ $F_{iCO_2} = 0.0005$), Gas 2 was drawn from a different contained gas cylinder ($F_{iO_2} = 0.07$ $F_{iCO_2} = 0.05$). Prior to the commencement of each test software automated further sampling of the ambient environment to ensure accuracy.

Repeatability data for both Douglas Bag analysis and gas analysis using the MetaMax3X is presented in experimental study 1 (Chapter 4) and thus will not be presented in this section.

3.13.3 CALCULATIONS DERIVED FROM CARDIOPULMONARY MEASURES

Equation 3.2 Calculation of Respiratory Exchange Ratio (RER) (Bergman et al., 1999; Lusk, 1923)

$$\text{Respiratory Exchange Ratio} = \dot{V}CO_2 / \dot{V}O_2$$

Equation 3.3: Calculation of oxidation rates of fat and carbohydrate (CHO) (Lusk, 1923)

$$\text{CHO (g.min}^{-1}\text{)} = (4.585 \times \dot{V}\text{O}_2) \text{ (L.min}^{-1}\text{)} - (3.226 \times \dot{V}\text{CO}_2) \text{ (L.min}^{-1}\text{)}$$

$$\text{Fat (g.min}^{-1}\text{)} = (1.695 \times \dot{V}\text{O}_2) \text{ (L.min}^{-1}\text{)} - (1.701 \times \dot{V}\text{CO}_2) \text{ (L.min}^{-1}\text{)}$$

3.13.4 PERIPHERAL ARTERY OXYGEN SATURATION

Peripheral arterial oxygen saturation (SpO_2) was estimated, using a finger pulse oximeter (Nonin 2500, Nonin Medical Inc, USA). Values were recorded after a period of 10 seconds of wearing the pulse oximeter to allow for stability of the readings. Peripheral arterial oxygen saturation values were recorded every 5 minutes during hypoxic exposure in the hypoxic chamber. Peripheral oxygen saturation can be calculated with pulse oximetry using the following formula;

$$SpO_2 = \text{HbO}_2 / \text{HbO}_2 + \text{Hb}$$

Pulse oximetry uses a light emitting diode in conjunction with a light sensitive sensor to measure absorption of red and infra-red light in the extremity. Differences in absorption between oxygenated and deoxygenated haemoglobin exists allowing for the calculation to provide an estimate.

3.13.5 HEART RATE

Heart rate was recorded using a Polar HR monitor (Polar Electro Oyo, Kempele, Finland). A chest strap transmitter was securely affixed to the participant and the sensors dampened to aid conductivity. The wrist watch receiver was also worn to enable live HR data recording. According to the manufacturer's instructions the HR monitor is accurate during steady state conditions to $\pm 1\%$ or ± 1 beats.min⁻¹, whichever is larger. Throughout all trials HR was recorded every 5 minutes during hypoxic exposure in the hypoxic chamber.

3.14 PERCEPTUAL SCALES

LAKE LOUISE QUESTIONNAIRE SCORE

A Lake Louise Questionnaire (Sampson et al., 1983; Savourey et al., 1995) (Figure 3.1) was implemented during acute hypoxic exposures. Symptoms of acute mountain sickness (AMS) were calculated from the sum of four questions (0-3), including headache, gastrointestinal upset, fatigue or weakness, and dizziness or light-headedness, the quality of sleep question was extracted due to irrelevance for acute daytime exposures. Scores of >5 have been suggested to represent the presence of AMS although this is not widely agreed (Bartsch et al., 2004) and is also inclusive of sleep based questions. Other studies recommend a score of >5 as a recommendation (Hackett and Roach 2001) which was employed in this thesis.

Lake Louise Score (LLS) for the diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:

1. A rise in altitude within the last 4 days
 2. Presence of a headache
- PLUS**
3. Presence of at least one other symptom
 4. A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE

Add together the individual scores for each symptom to get the **total score**.

Headache	No headache	0	
	Mild headache	1	
	Moderate headache	2	
	Severe headache, incapacitating	3	
Gastrointestinal symptoms	None	0	
	Poor appetite or nausea	1	
	Moderate nausea &/or vomiting	2	
	Severe nausea &/or vomiting	3	
Fatigue &/or weakness	Not tired or weak	0	
	Mild fatigue/ weakness	1	
	Moderate fatigue/ weakness	2	
	Severe fatigue/ weakness	3	
Dizziness/lightheadedness	Not dizzy	0	
	Mild dizziness	1	
	Moderate dizziness	2	
	Severe dizziness, incapacitating	3	
Difficulty sleeping	Slept as well as usual	0	
	Did not sleep as well as usual	1	
	Woke many times, poor sleep	2	
	Could not sleep at all	3	
TOTAL SCORE:			

Total score of:

- 3 to 5 = mild AMS
- 6 or more = severe AMS

Note:

- Do not ascend with symptoms of AMS
- Descend if symptoms are not improving or getting worse
- Descend if symptoms of HACE or HAPE develop

FIGURE 3.1: Lake Louise questionnaire score (Sampson et al., 1983).

3.14.2 RATING OF PERCEIVED EXERTION

The rating of perceived exertion scale (RPE), also known as the Borg scale (Borg, 1962), is a psychophysical tool to assess subjective perception of effort during exercise. Rating of perceived exertion ranged from 6 (very, very light), through 13 (somewhat hard), to 20 (very, very hard) along a 15-point scale (Figure 3.2). Rating of perceived exertion has been reported to be closely related to both metabolic and cardiac intensity parameters (Scherr et al., 2013). Participants were provided with standardised instructions that provided specific commands on how to report overall feelings of exertion throughout the experimental trials. The standardised instructions included clear understanding of anchoring the top and bottom ratings to previous experiences of no exertion at all

(RPE = 6) and maximal exertion (RPE = 20) (Mauger et al., 2013). Within this thesis, RPE was recorded at 10 minute intervals throughout the cycling sessions.

RATING OF PERCEIVED EXERTION	
6	
7	Very, very light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

FIGURE 3.2 Rating of perceived exertion (RPE) scale (Borg et al., 1985).

3.15 STATISTICAL ANALYSIS

All data was analysed using a standard statistical package (SPSS version 20.0) and were reported as mean \pm standard deviation (SD). Statistical significance was accepted at the level of $p \leq 0.05$.

3.15.1 DESCRIPTIVE STATISTICS

3.15.1.1 MEASUREMENT OF CENTRAL TENDENCY - MEAN

Within this thesis, the mean is reported to describe central tendency of each individual data set. The mean is calculated as the sum of the values divided by the number of values as is expressed as the unit measured.

Equation 3.4: Calculation of the mean

$$\text{Mean} = (\Sigma xi) / n$$

Where Σ = the sum, xi = the data, n = the number of data points within the analysis

3.15.1.2 VARIATION OF DATA

Statistical variance describes the spread of numbers within a data set with a variance of zero indicating all the values are identical. Small variance indicates that the data is distributed closely to the mean, a high variance indicates that the data are more greatly distributed away from the mean and from each other (Field 2013).

3.15.1.3 STANDARD DEVIATION

Standard deviation was used within this thesis to quantify the amount of variation or dispersion of each data set (Bland and Altman 1996). Standard deviation was calculated based upon the square root of the variance. For this thesis one standard deviation, expressed in the same units as the data, was reported thus assuming description of 68.2% of the normal distribution (Field 2013).

Equation 3.5: Calculation of standard deviation (SD)

$$SD = \sqrt{[\Sigma (xi - x)^2 / (n - 1)]}$$

Where Σ = the sum, xi = the data, x = the mean of the data set under analysis, n = the number of data points within the analysis

3.15.2 NORMALITY AND SPHERICITY OF DATA

3.15.2.1 NORMAL DISTRIBUTION

Data was checked for skewness and kurtosis to determine whether the distribution of scores was approximately normal. If values of skewness and kurtosis were between -1.96 and 1.96 data was assumed normally distributed (Field, 2013). Furthermore, the Shapiro-Wilk method was used to compare the scores in the data set to a normally distributed set of data with an identical mean and SD. If the test was not significant (i.e. $p \geq 0.05$), then the distribution was deemed not significantly different from the normal distribution and data was assumed normally distributed (Field, 2013).

3.15.2.2 SPHERICITY

Sphericity was assessed using the Mauchly's test, which tests the hypothesis that the variance of the differences between conditions is equal. If Mauchly's test statistic was significant ($p \leq 0.05$), it was concluded that there were significant differences between the variances of differences and therefore, the condition of sphericity was not met. If the assumption of sphericity was not met the degrees of freedom were adjusted using the Greenhouse-Geisser method. If Mauchly's test statistic was non-significant ($p \geq 0.05$) then it was concluded that the variance of differences were about equal (Field, 2013).

Equation 3.6: Calculation of skewness

$$\text{Skewness} = \Sigma Z^3 / n$$

Where Σ = the sum, Z = z-score of the data, n = the number of data points within the analysis

Equation 3.7: Calculation of kurtosis

$$\text{Kurtosis} = (\Sigma Z^4 / n) - 3.0$$

Where Σ = the sum, Z = z-score of the data, n = the number of data points within the analysis

Acceptable limits of skewness and kurtosis within this thesis have been set within the range of -2 to + 2

3.15.3 STATISTICAL TESTS OF DIFFERENCE

3.15.3.1 STATISTICAL SIGNIFICANCE LEVEL

Within this thesis a statistical significance level was set to describe the probability (p) that an analysis of data as the observed sample would be generated under a model of random chance. The threshold p value for all experiments within this thesis was set at 5% thus, giving 95% confidence that there was an actual difference or association, rather than a chance difference or association. Within this thesis, a p value ≤ 0.05 indicates that the observed effect is unlikely to have arisen purely by chance, providing evidence against the null hypothesis. Furthermore, a p value ≥ 0.05 resulted in the alternative hypothesis being rejected and it was concluded that the effect was not big enough to be found. Two-tailed test of differences were carried out throughout the thesis due to the unknown direction of difference between samples.

3.15.3.2 EFFECT SIZES

In this thesis effect sizes reported are Cohen's d and partial eta squared (ηp^2). The recommendations for these calculations are based upon the review of Lakens (2013).

3.15.3.3 COHEN'S D (d)

Cohen's D (d) was used to describe the standardised mean difference of an effect for between-subjects designs as it is directly related to a t-test (Lakens 2013). Interpretation of d was made in accordance with the following where the effect size was identified as small ($d = 0.2$), medium ($d = 0.5$) and large ($d = 0.8$) based on established benchmarks (Cohen 1988).

Equation 3.8: Calculation of Cohen's D (d)

$$d = (x^1 - x^2) / SD$$

Where x^1 is the mean of the first data set under analysis and x^2 is the mean of the second data set under analysis. SD is standard deviation.

3.15.3.4 PARTIAL ETA SQUARED (np^2)

Partial eta squared (np^2) was preferred to eta squared (η^2) to improve the comparability of effect sizes between groups/ studies (Lakens 2013), notably those using analysis of variance (ANOVA). np^2 expresses the sum of squares of the effect in relation to the sum of squares of the effect and the sum of squares of the error associated with the effect. Interpretation of np^2 was made in accordance with the following where the effect size was identified as small ($np^2 = 0.01$), medium ($np^2 = 0.06$), and large ($np^2 = 0.13$) based on established benchmarks (Cohen 1988).

Equation 3.9: Calculation of partial eta squared (np^2)

$$np^2 = SS_{\text{effect}} / (SS_{\text{effect}} + SS_{\text{error}})$$

Where SS_{effect} is the sum of squares of the effect, and SS_{error} is the sum of squares of the error. Both of these are taken from the ANOVA performed.

3.15.3.5 ANALYSIS OF VARIANCE (ANOVA)

Analysis of variance (ANOVA) describes a collection of statistical models used in order to analyse the differences between group means and their associated procedures (such as "variation" among groups) (Field, 2013). More precisely ANOVA is a way of comparing the ratio of systematic variance to unsystematic variance in an experimental study. The ratio of these variances is known as the F -ratio. Within this thesis one way and two way ANOVA have been implemented to test within groups with repeated measures. Within the statistical analysis section of each experimental chapter the type of ANOVA (s) used for each data set are described in detail.

One-way ANOVA was used to compare means of three or more samples (using the F distribution) (Vincent 1999). Two-way ANOVA was implemented as an extension of the one-way ANOVA to examine the influence of two different categorical independent variables on one continuous dependent variable thus assessing the main effect of each independent variable but also whether any interaction effect has occurred between them (Vincent, 1999).

Data entered for ANOVA was assumed to meet the previously described criteria for distribution, normality and equality. The calculated f statistic (f) for each ANOVA comparison was referenced to the corresponding degrees of freedom with statistical significance level of $p = 0.05$ accepted.

3.15.3.6 POST-HOC TESTING

Bonferroni corrections were utilised as post-hoc analysis due to their conservative estimation of difference between multiple comparisons (Field, 2013). The Bonferroni correction corrects the p value of the t tests based on the number of comparisons that are being made. The Bonferroni correction is calculated by dividing 0.05 by the number of comparisons that are being made. The conservative application of the Bonferroni correction is derived from experimental designs testing dependent or independent hypotheses. Under these conditions maintenance of the familywise error rate is facilitated by testing each individual hypothesis (Field, 2013).

3.15.4 RELIABILITY STATISTICS

3.15.4.1 TYPICAL ERROR OF MEASURE (TEM)

Within this thesis the TEM was calculated to represent the absolute repeatability of measurements used. TEM was calculated from the SD of the difference score by the square root of two (Hopkins, 2000).

Equation 3.10 Calculation of typical error of the measure (TEM) (Norton et al. 1996)

$$\text{TEM} = \text{SD}_{\text{diff}} / \sqrt{2}.$$

Where: SD_{diff} = standard deviation of the difference between the repeated measures; $\sqrt{2}$ = square root of 2.

3.15.4.2 COEFFICIENT OF VARIATION

Within this thesis the TEM has been expressed as a percentage of its respective mean to form the technical error as a coefficient of variation (TE (CV %)) (Hopkins, 2000).

Equation 3.11 Calculation of technical error as a coefficient of variation (TE (CV %))

$$\text{TE (CV \%)} = (\text{TEM} / \text{Mean}) * 100$$

Where: TEM = typical error of measure, Mean = the mean of the mean of the paired samples

3.15.4.3 INTERCLASS CORRELATION COEFFICIENT

Within this thesis ICC with 95% CI were calculated using SPSS as a measure of retest correlation. Prior to conducting this test data was first checked for normality as previously described.

Equation 3.12 Calculation of intra class correlation (ICC)

$$\text{ICC} = (\text{SD}^2 - \text{sd}^2) / \text{SD}^2$$

Where: SD = between subject standard deviation, sd = within subject standard deviation

3.16 POWER ANALYSIS

The number of participants required for each experimental chapter was determined using G*Power version 3.1 in accordance with established guidelines for *a priori* determination (Prajapati et al., 2010). The value of α and β were set as 0.05 and 0.8, respectively.

3.17 PHLEBOTOMY AND BIOCHEMISTRY

3.17.1 CANNULATION

Prior to blood sampling all participants were placed in a lying position and an appropriate arm was chosen and cleaned with alcohol wipes. A tourniquet was used to select a vein and allow the insertion of a sterile cannula (18G I.V. Catheter, Biovalve, Vygon, UK). After insertion of the cannula the tourniquet was removed and the cannula was then flushed with 10ml of saline solution (0.9% Sodium Chloride I.V. Infusion, Baxter Healthcare LTD, Norfolk, UK). Blood (10ml) was taken via the cannula from the major vein located in the ante cubital fossa during each experimental trial. A 10ml syringe (BandD Plastipak 10ml Syringe, Hamburg, Germany) was used to draw the blood. A further 10ml of saline was then used to flush the cannula after each sample was taken, equal to the amount of blood extracted. At the end of each trial the cannula was removed with the location of the insertion compressed using tissue until bleeding ceased.

3.17.2 BLOOD PLASMA SEPARATION

Collected whole blood was immediately transferred into two 5ml tubes containing ethylenediaminetetraacetic acid (EDTA: 32.332 Sarstedt, Akteingesellschaft and Co, Numbrecht, Germany). Whole blood samples were centrifuged (Eppendorf 5804 R Centrifuge) at 5000rpm for a period of 10 minutes at 5°C to separate plasma for appropriate analysis. Blood plasma was separated via pipetting (Eppendorf Research/Research Pro) into 2ml micro-tubes (Eppendorf) and stored at -86°C (Sanyo Ultra Low, VIP Series) until analysis. Typically three aliquots per sample point were stored for analysis.

3.17.3 ANALYSIS OF FFA

The Wako NEFA C test kit utilises an *in vitro* enzymatic calorimetric method for the quantification of non-esterified (free) fatty acids in plasma. The intensity of the red pigment is proportional to the concentration of FFA in the sample with absorbance measured at a wavelength 550nm, with 1cm light path. The ready to use colour reagent solutions A and B were prepared by mixing the provided solvents with 10mL of colour reagent A and 20mL of colour reagent B respectively. Once mixed all solutions were sheltered from light, kept refrigerated at 4°C and used within the recommended five day shelf life. Colour reagent A's components include Acyl-CoA-synthetase, Ascorbate oxidase, Coenzyme A, adenosine triphosphate, 4-Aminophenazone and Sodium Azide. Colour reagent B's components include Acyl-CoA-Oxidase and Peroxidase. Solvent A's components include Phosphate buffer,

Magnesium Chloride, Surfactant and Stabilisers. Solvent B's components include MEHA and Surfactant.

75uL of colour reagent A was added and mixed with 10uL sample in duplicate before being incubated for 10 minutes at 37°C prior to 150uL of colour reagent B being added, mixed and incubated for a further 10 minutes at 37°C. Absorbance was then read using a micro plate reader (550nm, 1cm) (ELx 800 Universal Micro plate Reader, Bio-Tek Instruments, USA). Precision of the Wako NEFA C has been established reporting a CV of 2.7% when analysing a series of standards in replicate. Duplicate samples were analysed for each assay. An intra-assay CV of 2.3% for FFA was calculated.

3.17.4 ANALYSIS OF TAG

L-Type Triglyceride M is an in vitro assay for the quantitative determination of triglyceride in plasma utilising N-(3-sulfo-propyl)-3-methoxy-5-methylaniline (HMMPs) that produces blue pigment in an enzymatic method. The amount of triglycerides contained in the sample is determined by measuring the absorbance of the blue colour. Triglycerides in the sample were hydrolysed to glycerol and FFA in a reaction catalysed by lipoprotein lipase the glycerol before being converted to glycerol-3-phosphate by glycerol kinase in the presence of ATP. Glycerol-3-phosphate was then oxidised by glycerol-3-phosphate oxidase in a reaction that produces hydrogen peroxide which subsequently causes the production of a blue pigment. 2uL of sample were pipetted in duplicate in to the 96 well plate before 300uL of colour reagent was added, mixed and incubated at 37°C for 5 minutes. Absorbance was then read using a micro plate reader (600nm, 1cm) (ELx 800 Universal Micro plate Reader, Bio-Tek Instruments, USA). Duplicate samples were analysed for each assay. An intra-assay CV of 3.3% for TAG was calculated.

3.17.5 ANALYSIS OF METEORIN-LIKE (METRNL)

Meteorin-like was analysed using a quantitative sandwich enzyme immunoassay technique (Cusabio, China) with an intra-assay precision of CV% < 8%, an inter-assay precision of CV% < 10% and a detection range of 0.156 ng/ml – 10 ng/ml. An Antibody specific for Meteorin-like had been pre-coated onto a microplate. In accordance with the detection range of the assay; eight standards with concentrations of 0, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 ng/ml were prepared. Standards and samples were pipetted into the wells and any Meteorin-like present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Meteorin-like was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and colour was developed in proportion to the amount of Meteorin-like bound in the initial step. Absorbance was then read using a micro plate reader (450nm, 1cm) (ELx 800 Universal Micro

plate Reader, Bio-Tek Instruments, USA). Duplicate samples were analysed for each assay. An intra-assay CV of 3.6% for Meteorin-like was calculated.

Following ELISA plate analysis data was analysed using a standard curve fitting as per assay instructions. Duplicate readings for each standard and sample were averaged. The average standard zero was then subtracted from the standard and sample values. A standard curve was constructed by plotting the mean absorbance of each standard on the x-axis against the concentration on the y-axis with a best fit curve drawn through the points on the graph. Meteorin-like data values were then calculated based on the slope and intercept of the standard curve.

LIST OF STUDY CHAPTERS

CHAPTER 4

STUDY 1: Examination of the reproducibility and validity of the MetaMax3X in normobaric hypoxia at rest

CHAPTER 5

STUDY 2: An acute exposure to $FiO_2:0.12$ induces alterations in markers of lipid metabolism in a healthy human population

CHAPTER 6

STUDY 3: The separate and combined effects of acute hypoxia, moderate intensity exercise and lipid ingestion on markers of metabolism

CHAPTER 7

STUDY 4: Moderate altitude induces metabolic effects in healthy humans acutely and following an 18 day stay at 3,400m

CHAPTER 8

STUDY 5: The effects of a moderate altitude stay on postprandial metabolic parameters

4. EXAMINATION OF THE REPRODUCIBILITY AND VALIDITY OF THE METAMAX3X IN NORMOBARIC HYPOXIA AT REST

4.1 ABSTRACT

Aim: The use of portable metabolic analysers is commonplace (Macfarlane 2001). Such systems are able to provide more data than the traditional Douglas bag method with analysis in real time situations possible. Few independent studies, however, have been published concerning the validity and reproducibility of such gas analysers and fewer for use in hypoxia or non-sea level experiments. The present study therefore aimed to examine the reproducibility and validity of the Cortex MetaMax3X at rest in normobaric hypoxia (F_{iO_2} : 0.125). **Method:** Testing was performed on three separate occasions. Reproducibility was obtained from duplicate sessions. Validity was determined by the comparison of results against a gold standard Douglas bag. Trials were randomised for order and completed at rest by healthy volunteers ($n=9$; 7 males, 2 females) 24 ± 4 years of age (mean \pm SD), body mass 77 ± 10 kg, height 178 ± 10 cm. **Results:** Repeated MetaMax3X trials yielded no differences in measures of $\dot{V}O_2$, $\dot{V}CO_2$, RER and $\dot{V}E$. The MetaMax3X reported greater values of $\dot{V}O_2$ (+ 0.03 L.min⁻¹ $p = 0.000$), $\dot{V}CO_2$ (+ 0.02 L.min⁻¹ $p = 0.001$) and $\dot{V}E$ (+0.6 L.min⁻¹ $p = 0.014$) and lesser values of RER (-0.07 $p = 0.002$) at rest compared to the Douglas Bag method in normobaric hypoxia. **Conclusions:** These results indicate that the MetaMax3X has levels of reproducibility that are comparable to previous investigations. Variability in measures at rest between the MetaMax3X and Douglas Bag suggests that there should be caution in interpreting the values obtained by the MetaMax3X in normobaric hypoxia. If used, reported values of $\dot{V}O_2$ and RER in the current investigation by the MetaMax3X may lead to errors in estimates of EE of 25 kcal.hr⁻¹ greater at rest and 40% increases in fat utilisation compared to values obtained by the Douglas bag method.

4.2 INTRODUCTION

The Douglas bag method for indirect calorimetry has been considered a “gold standard” in providing an accurate assessment of expired air and calculation of metabolic function for over a century (Bassett et al., 2001; Brehm et al., 2004). The method has robust procedures and analytical precision and has been well tested to measure human performance under various conditions (Astrand and Rosdahl.1986; Rosdahl et al. 2010). It has, however, been suggested that the method carries some limitations (Crouter et al., 2006). The inability to measure rapid changes in ventilation, the time consuming nature of the post-measurement gas analysis (Rosdahl et al. 2010; Carter and Jeukendrup 2002), the slight gas permeability of the bag material and the nature of the collection method resulting in the contents of the bag being representative of the entire sampling period, all highlighted as potential limitations for the method (Hodges et al., 2005).

The use of portable metabolic analysers is now commonplace (Macfarlane 2001) as such systems are able to provide more information, particularly acute responses, than the traditional Douglas bag method, however the application of such analysers in a variety of environmental conditions has not been validated. The need for further analysis of possible systematic errors in such analysers, to facilitate greater consistency of measurement and reliability has been emphasised (Rosdahl et al., 2010). Few independent studies have been published concerning the validity and reproducibility of portable gas analysers (Hodges et al., 2005) and less so for their use in hypoxia or non-sea level experiments. The Douglas Bag method has been suggested as the best reference system for comparison of online metabolic analysers (Prieur et al., 2003). Previous research in which the Douglas bag method was used as criterion have reported over and under estimations in $\dot{V}O_2$ (Miodownik et al., 2000) $\dot{V}CO_2$, RER (Engelbretson 1998), and \dot{V}_E (Carter and Jeukendrup 2002) when compared with metabolic respiratory systems. A number of reasons have been highlighted as possible contributing factors for differences found in metabolic and pulmonary measures reported in previous investigations. Such reasons relate to both a lack of consistency in experimental study designs from one study to another and factors associated with the metabolic analysers themselves. A comprehensive review from Hodges et al., (2005) outlined the metabolic analyser factors as differences in software to acquire information, differing analyser times, and the presence of water vapour in the analyser as some of the main determinants of differences found when comparing metabolic analysers with each other and with criterion methods. With regards to experimental design a lengthier list of reasons were highlighted in the same review most notably; a lack of consistency in the exercise protocol and exercise mode administered, differences in what are accepted as acceptable limits of gas measurements, a lack of reporting with relation to the timing of the taken measurements, differences in the statistical analysis of the data and a lack of consistency in the manner in which measurements are made on the systems being compared. The final point referring to some measures being made in parallel, as recommended, compared to studies in which repeated measures or in series measurements were employed.

The MetaMax3X (Cortex, Leipzig, Germany) is a commercially available mixed chamber gas analysis system; independent research investigating the validity of this equipment is scarce. Studies from Larsson et al. (2004) and Medbo et al. (2002) using earlier versions of the MetaMax system (MetaMax I and II) have provided some reference values and criteria for measurements during exercise. Medbo et al. (2002) found that O₂ uptake is measured precisely within subjects by the MetaMaxes, however there are systematic errors and variations between subjects when comparing against the Douglas Bag method during exercise.

A recent investigation (Vogler et al., 2010), assessing both the validity and reproducibility over five differing exercise intensities of the same system, reported that in terms of reproducibility the MetaMax3B yielded typical errors of 2-3% for measures of VO₂, VCO₂ and V_E and is therefore at least comparable to that of the Douglas bag system. Although the MetaMax3B provided a satisfactory indication of the actual metabolic demand of an activity, the reported results suggest that the measurements were not entirely comparable with criterion systems, with overestimates of VO₂ (+4%), VCO₂ (+7%) and V_E (+4%).

Review articles concerning online metabolic analysers (Hodges et al. 2005; Macfarlane 2001) have highlighted similar variations in many systems. In the majority of cases differences are considered to be small and not significant for interpretation of the data. Modern metabolic analyser systems, when compared with the Douglas bag method and other metabolic simulators, may provide errors by over or underestimating ventilatory volumes by up to 10.5% and significantly more errors are found when ventilation rates are higher compared to when they are lower (Hodges et al., 2005), likely due to the delay in the sampling and measurement of gases. High ventilation rates, elicited by hypoxia or exercise, may also cause errors of measurement when such equipment is used (Hodges et al., 2005).

Oxygen supply is known to be one of the environmental conditions that may affect resting EE (Oltmanns et al., 2006). Exposure to high and extreme altitudes (5,300m – 8,848m), where hypoxia and reduced blood oxygen saturation occurs, have been demonstrated to elicit adaptations and acclimation to such atmospheric conditions and a steady reduction in weight with increasing severity of hypoxia (Reynolds et al., 1999). Due to a wide range of research interest, from sport to health applications, the ability to measure metabolic consequences and respiratory compensation would be easier and provide useful evidence for adaptive or maladaptive changes if online systems were shown to be reliable and accurate in such conditions. Studies in recent years have highlighted the possibility of using hypoxic exposures as a tool to aid weight loss in obese populations (Lippl et al., 2010; Netzer et al., 2008). Measures of EE by indirect calorimetry may be important in assessing changes in physiological processes that regulate EE under hypoxic conditions. Given the potential scientific benefits for measuring gas exchange and metabolism during normobaric hypoxia in real time and to assess the equipment for future planned research in the field at altitude the primary aims of the study were to 1) Investigate the reproducibility of the MetaMax3X in normobaric hypoxia at rest. 2)

Investigate the validity of the MetaMax3X in normobaric hypoxia at rest. It was hypothesised that the MetaMax would produce reproducible measures of metabolic markers during exposure on repeated occasions to normobaric hypoxia at rest. It was also hypothesised that the MetaMax 3X will display good agreement with the gold standard Douglas bag method of collection. This present study sits within the overall aim of the thesis by providing information regarding the validity and reproducibility of the MetaMax3X which is used at a later stage to measure markers integral to the thesis aims.

4.3 MATERIALS AND METHODS

PARTICIPANTS

Healthy volunteers (n=9: 7 males, 2 females) 24 ± 4 years of age (mean \pm SD), body mass 77 ± 10 kg, height 178 ± 10 cm and BMI 24 ± 2 (kg/m^2) agreed to partake in the study and complied with all criteria for participation (3.5.1), medical criteria and recruitment, (3.6) pre-trial diet and exercise standardisation, and (3.8) Hydration assessment. Prior to the undertaking of the experimental trials volunteers attended the laboratories whereby their anthropometric data was collected [(3.11)].

EXPERIMENTAL DESIGN

The study required the participants to attend the laboratory on three occasions at the same time of day. Participants were exposed to FiO_2 : 0.125 in a purpose built hypoxic chamber for a period of 45 minutes following a period of 30 minutes rest in normoxic conditions (FiO_2 : 0.21) on each trial. The chamber environment was controlled for each trial [(3.12.2) Controlling the hypoxic environment]. FiCO_2 values were also recorded as the environment was a closed system. Gas exchange measurements were conducted with the MetaMax3X during two trials to establish the reproducibility of the results, with a single trial being completed using the Douglas Bag system in order to provide a criterion against which the validity of the MetaMax3X could be compared. The participants were instructed to remain quiet in a supine position during data collection but to remain awake. Simultaneous MetaMax3X and Douglas Bag measurements were unable to be taken due to the nature of the MetaMax3X collection and its calibration process. The trials were randomised for order. Each trial was separated by one week in order to avoid any acclimatisation effects of hypoxic exposure.

METAMAX3X

The MetaMax3X (Cortex Medical, Leipzig, Germany) is a portable cardio pulmonary exercise testing (CPET) system that works in accordance with the mixed chamber measuring technique. The system records the oxygen and carbon dioxide content in expired air along with the ventilation and heart rate of an individual, sampling at a 10 second average. This information is then transferred to a PC where it is analysed using the software package (Metasoft, Cortex Medical, Leipzig, Germany). Collected data is stored but can also be transmitted to a telemetry receiver for instantaneous display.

The system was secured to the chest with a harness made up of a battery and two housing shells, which contains the sensors and memory device. Expired volume was measured using a turbine sensor (Triple V Turbine, digital). O₂ concentration was measured with an electrochemical cell whereas CO₂ was measured by an ND infrared laser. The device also contained a temperature and atmospheric pressure sensor. Respiratory values were determined through the use of the Haldane transformation (Haldane 1912; Haldane 1922). The Haldane transformation is used to calculate Oxygen uptake by pulmonary gas exchange when only expiratory or inspiratory ventilation is used (Poole and Whipp, 1988). As such $\dot{V}O_2$ was calculated using the following equation; $\dot{V}O_2 = (V_i \times FiO_2) - (V_E \times FeO_2)$ where; $\dot{V}O_2$ equals the volume of Oxygen, V_i equals the volume of inspired air, FiO_2 equals the fraction of inspired Oxygen, V_E equals the volume of expired air and FeO_2 equals the fraction of expired Oxygen. Carbon dioxide production ($\dot{V}CO_2$) was calculated using the following equation; $\dot{V}CO_2 = (V_E \times FeCO_2) - (V_i \times FiCO_2)$ where; $\dot{V}CO_2$ equals the volume of Carbon Dioxide, V_E equals the volume of expired air, V_i equals the volume of inspired air, $FiCO_2$ equals the fraction of inspired Carbon Dioxide and $FeCO_2$ equals the fraction of expired Carbon Dioxide. Furthermore V_i is calculated using the following equation; $V_i = V_E \times FeN_2 / FiN_2$ where; FeN_2 and FiN_2 equal the fractional concentrations of nitrogen in the expired and inspired air, respectively. The assumption that the volume of inspired and expired Nitrogen are equal is applied within the Haldane transformation. Accuracy of the sensors according to the manufacturers guidelines are reported as; (coefficient of variation) $\pm 2\%$ volume, $\pm 0.1\%$ volume for $\dot{V}O_2$ and $\dot{V}CO_2$, $\pm 1^\circ C$ for temperature and $\pm 1.8\%$ for pressure.

CALIBRATION OF THE METAMAX3X

The MetaMax3X was calibrated according to normal operational procedures for hypoxic conditions before each set of measurements.

GAS AND PHYSIOLOGICAL MEASURES

During the Douglas Bag trial expired gas was collected into Douglas bags over approximately 60 seconds every 5 minutes during exposure to normobaric hypoxia. Inspired O₂ and CO₂ fractions were monitored constantly using a sample tube connected directly to a gas analyser (Servomex 1400, Servomex Group Ltd., England), the analyser was calibrated prior to each experiment.

Peripheral arterial oxygen saturation was measured (SpO_2), using a finger pulse oximeter (Nonin 2500, Nonin Medical Inc., USA) [(3.13.4) Peripheral artery oxygen saturation)], and heart rate (HR) using a HR monitor (Polar, Finland) was also monitored [(3.13.5) Heart rate]. A heart rate monitor was used in order to keep the recording of heart rate consistent across all three trials.

DATA ANALYSIS

During the Douglas bag trial expired gas was collected during the fifth minute of each five minute interval to determine $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER. Values for the MetaMax3X trials were determined by averaging all values acquired during the fifth minute of each five minute interval. An approximate 60 seconds worth of expired air was collected at five minute intervals and used for analysis as this was determined to provide sufficient data to address the research question as well as being practically effective with the time and equipment constraints. A 60 second data collection has also been conducted in a study previously in which similar values were observed with a mixed chamber gas system highlighting this collection period as suitable for measures of $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER (Nieman et al., 2013). The method of MetaMax3X analysis was conducted in an attempt to best replicate the aforementioned Douglas bag method as to compare 60 second measurements across collection methods.

STATISTICAL ANALYSIS

All data were checked for normality and sphericity [(3.15.1.2) Normal distribution and (3.15.2.2) Sphericity]] and were adjusted using the Huynh-Feldt method. All data were analysed using a standard statistical package (SPSS version 20 for Windows 7).

Reproducibility between duplicate MetaMax3X trials was described by typical errors (TEM) in both absolute and percentage terms for the means of the overall exposure. Coefficients of variation (CV%) were calculated for comparison between duplicate MetaMax3X trials and between MetaMax3X trial 1 and the Douglas Bag trial with 95% confidence intervals.

Results from matched MetaMax3X trials and single Douglas bag trial were examined using One way (trial) ANOVA factorial analyses of variance (ANOVA) with repeated measures (SPSS 20 for Windows; SPSS, Chicago, IL). A post-hoc Bonferoni pairwise comparison was carries out in order to determine at which time points the differences were seen. Paired sample T-tests were used to examine the differences between normoxic and hypoxic physiological measures with a statistical significance set at $p \leq 0.05$ for all analyses. Effect sizes (cohens d) were calculated to analyse the magnitude of the interaction (Lakens, 2013).

Bland-Altman plots were used to show the variability in the individual mean bias between duplicate MetaMax3X trials. This allowed for the mean bias and 95% prediction confidence intervals to be shown. Bland-Altman plots were also processed for bias between MetaMax3X trial 1 and Douglas Bag trial in order to permit a direct comparison with results from other validity studies.

Equation 4.1: Calculation of limits of agreement 95% confidence intervals.

$$\text{Upper confidence interval} = \text{Mean difference} + (\text{SDdiff} \times 1.96)$$

$$\text{Lower confidence interval} = \text{Mean difference} - (\text{SDdiff} \times 1.96)$$

Where *SDdiff* is the standard deviation of the difference values

4.4 RESULTS

No differences in FiO_2 (0.125 ± 0.003) ($F_{(2, 18)} = 0.049$, $p = 0.952$, $\text{np}^2 = 0.005$) were found between the three trials at normobaric hypoxia.

Hypoxia induced greater HR ($\text{b}\cdot\text{min}^{-1}$) ($t_{(3)} = -7.306$, $p = 0.005$, $d = 4.58$), \dot{V}_E ($\text{L}\cdot\text{min}^{-1}$) ($t_{(3)} = -7.611$, $p = 0.005$, $d = 5.79$), $\dot{V}\text{O}_2$ ($\text{L}\cdot\text{min}^{-1}$) ($t_{(3)} = -5.052$, $p = 0.015$, $d = 0.35$), $\dot{V}\text{CO}_2$ ($\text{L}\cdot\text{min}^{-1}$) ($t_{(3)} = -12.149$, $p = 0.001$, $d = 0.89$) and RER ($t_{(3)} = -10.554$, $p = 0.002$, $d = 3.73$) compared to pre-measures. A reduction in SpO_2 ($t_{(3)} = 20.822$, $p < 0.001$, $d = 15.19$) was also found. Resting values in normoxia and hypoxia are shown in Table 4.1.

TABLE 4.1: Mean \pm SD data for metabolic parameters in conditions of FiO_2 : 0.21 and FiO_2 : 0.12 measured by the Douglas Bag method.

FiO_2	$\dot{V}\text{O}_2$ ($\text{L}\cdot\text{min}^{-1}$)	$\dot{V}\text{CO}_2$ ($\text{L}\cdot\text{min}^{-1}$)	RER	\dot{V}_E ($\text{L}\cdot\text{min}^{-1}$)	HR ($\text{b}\cdot\text{min}^{-1}$)	SpO_2 (%)
0.2093	0.28 ± 0.01	0.22 ± 0.02	0.80 ± 0.06	6.6 ± 1.1	62 ± 1	98 ± 0
0.12	$0.35 \pm 0.02^*$	$0.33 \pm 0.01^*$	$0.95 \pm 0.07^*$	11.6 ± 0.4	$67 \pm 1^*$	$84 \pm 1^*$

Notes: Oxygen consumption ($\dot{V}\text{O}_2$), carbon dioxide production ($\dot{V}\text{CO}_2$), respiratory exchange ratio (RER), minute ventilation (\dot{V}_E), heart rate (HR $\text{b}\cdot\text{min}^{-1}$) and saturation of oxygen (SpO_2). * Denotes significant difference compared to the FiO_2 ; 2093 trial. All significance symbols correspond to $p < 0.05$.

REPRODUCIBILITY

Measures of reproducibility through repeated trials were assessed. No differences between trials in mean data were found for measures of $\dot{V}\text{O}_2$ ($F_{(1, 7)} = 0.063$, $p = 0.808$, $\text{np}^2 = 0.009$), $\dot{V}\text{CO}_2$ ($F_{(1, 7)} = 1.256$, $p = 0.214$, $\text{np}^2 = 0.016$), \dot{V}_E ($F_{(1, 7)} = 3.795$, $p = 0.06$, $\text{np}^2 = 0.089$) and RER ($F_{(1, 7)} = 0.059$, $p = 0.818$, $\text{np}^2 = 0.007$) ($p > 0.05$) with typical errors of $0.03 \text{ L}\cdot\text{min}^{-1}$ (7.52%), $0.03 \text{ L}\cdot\text{min}^{-1}$ (7.16%), $1 \text{ L}\cdot\text{min}^{-1}$ (8.25%) and 0.04 (4.51%) respectively.

VALIDITY

There was a difference between trials for measures of $\dot{V}O_2$ ($F_{(1,7)} = 35.29, p < 0.001, np^2 = 0.815$), $\dot{V}CO_2$ ($F_{(1,7)} = 18.134, p = 0.001, np^2 = 0.694$), RER ($F_{(1,7)} = 14.605, p = 0.002, np^2 = 0.646$), and \dot{V}_E ($F_{(1,7)} = 5.585, p = 0.014, np^2 = 0.411$). The MetaMax3X reported greater values for $\dot{V}O_2$ (+ 0.03 L.min⁻¹, $p = 0.001, d = 3.804$), $\dot{V}CO_2$ (+ 0.02 L.min⁻¹ $p = 0.03, d = 1.61$) \dot{V}_E (+0.6 L.min⁻¹, $p = 0.014, d = 1.84$) and lesser values of RER (- 0.07 $p = 0.011, d = 2.10$).

TABLE 4.2: Mean ± SD data, and reliability statistics for metabolic parameters using the MetaMax3X and the Douglas bag.

	$\dot{V}O_2$ (L.min ⁻¹)	$\dot{V}CO_2$ (L.min ⁻¹)	RER	\dot{V}_E (L.min ⁻¹)
MetaMax trial 1	0.45 ± 0.03	0.37 ± 0.03	0.81 ± 0.04	12.2 ± 0.9
MetaMax trial 2	0.45 ± 0.02	0.39 ± 0.02	0.87 ± 0.03	12.9 ± 0.9
CV	(6.9%)	(6.4%)	(5.4%)	(6.2%)
LOA	-0.00 (0.09. -0.1)	-0.04 (0.04. -0.11)	-0.07 (-0.03. -0.11)	-0.66 (2.21. -3.53)
Douglas Bag trial	0.35 ± 0.02*	0.33 ± 0.01*	0.95 ± 0.07*	11.6 ± 0.4*
CV	(6.2%)	(7.1%)	(2.2%)	(7.5%)
LOA	0.1 (0.18. 0.01)	0.02 (0.08. -0.04)	-0.16 (-0.02. -0.29)	0.61 (2.36. -1.14)

Notes: Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation (\dot{V}_E) and respiratory exchange ratio (RER). Mean coefficient of variation (CV) scores are displayed in brackets. The mean bias and 95% limits of agreement (LOA) for each variable are displayed. * Denotes significant difference compared to the MetaMax trial. All significance symbols correspond to $p < 0.05$.

Individual between-trial biases are displayed by Bland-Altman plots for MetaMax3X reliability in Figure 4.1. Bland Altman plots for MetaMax3X validity are displayed in Figure 4.2.

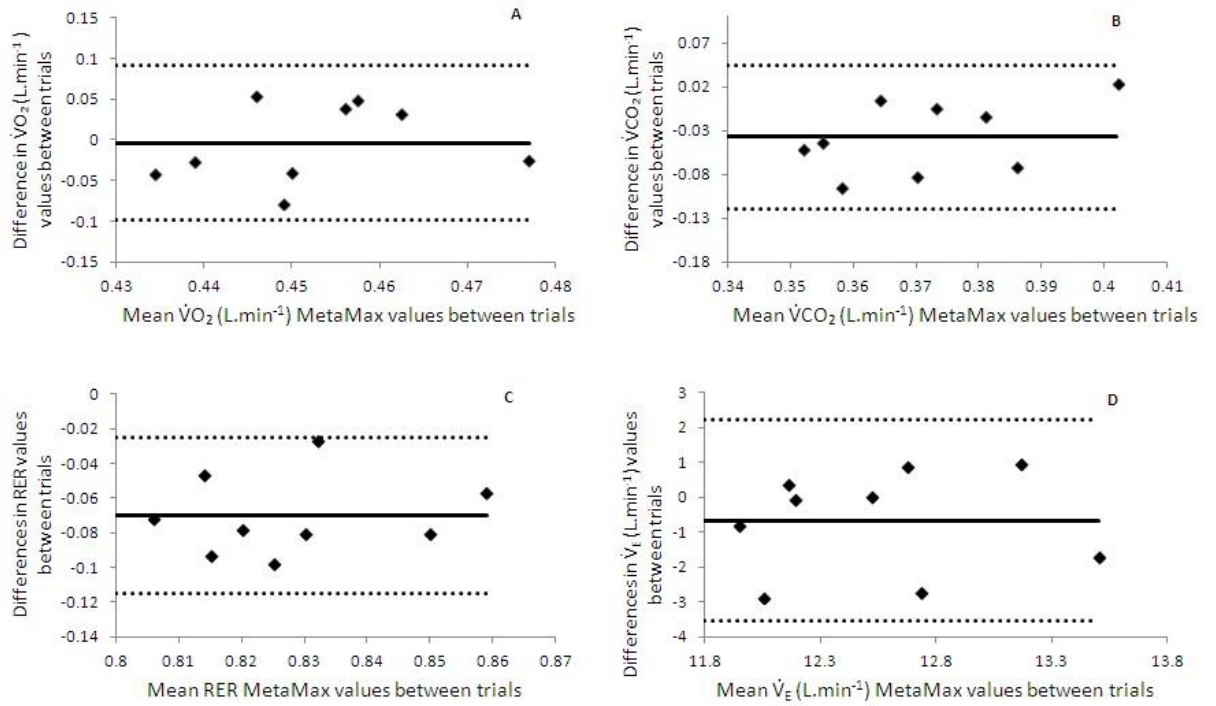


FIGURE 4.1: Bland-Altman plots of the mean bias (between MetaMax3X testing trials) for metabolic data.

Notes: The dashed lines indicate ± 1.96 SD. Oxygen consumption ($\dot{V}O_2$: A), carbon dioxide production ($\dot{V}CO_2$: B), minute ventilation (\dot{V}_E : C) and respiratory exchange ratio (RER: D).

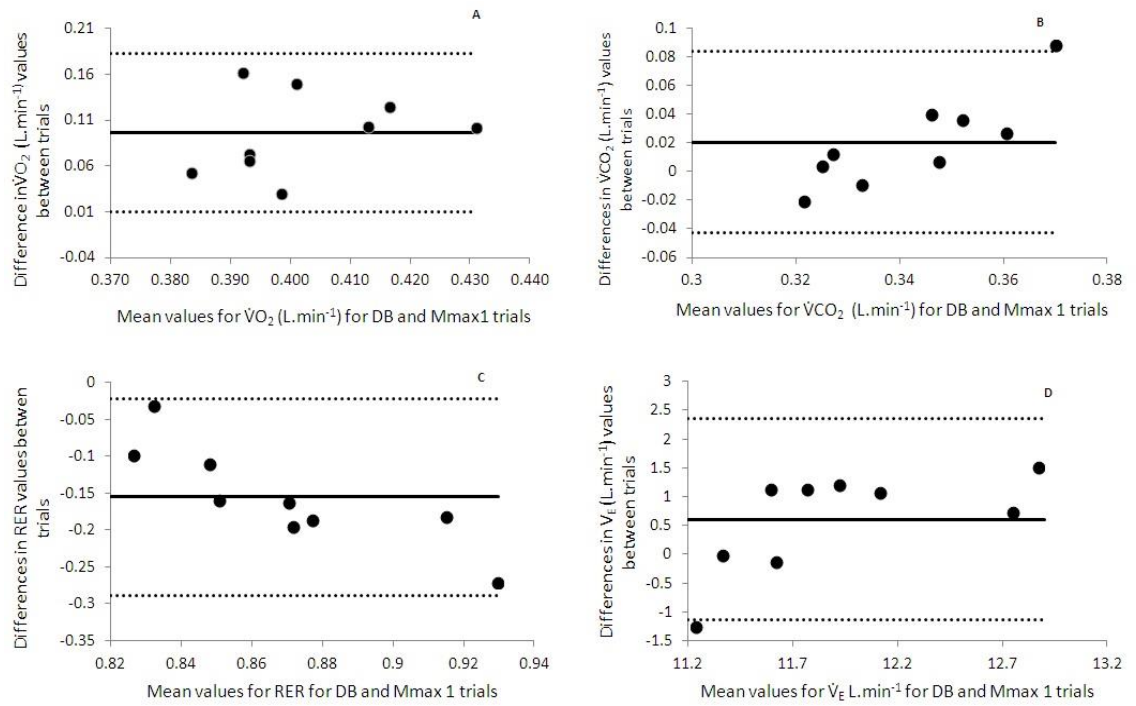


FIGURE 4.2: Bland-Altman plots of the mean bias (between MetaMax3X 1 and Douglas Bag (DB) testing trials) for metabolic data.

Notes: The dashed lines indicate ± 1.96 SD. Oxygen consumption ($\dot{V}O_2$: A), carbon dioxide production ($\dot{V}CO_2$: B), minute ventilation (\dot{V}_E : C) and respiratory exchange ratio (RER: D).

4.5 DISCUSSION

Information concerning the reproducibility and validity of online metabolic analysers in hypoxic conditions is limited. The purpose of the current study was to assess the reproducibility and validity of the Cortex MetaMax3X in normobaric hypoxic conditions at rest. Reproducibility measures indicated the MetaMax3X displayed no differences in $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER for repeated trials on the same individuals in normobaric hypoxia on two separate occasions. These results are consistent with previous studies (Carter and Jeukendrup, 2002; Crouter et al., 2010) in which the reproducibility of online systems have been seen to be within acceptable limits of measure. Validity measures indicated the MetaMax3X yielded significantly greater values for $\dot{V}O_2$, $\dot{V}CO_2$ and \dot{V}_E and significantly lesser values for RER, when compared to the Douglas Bag method during exposure to normobaric hypoxia (FiO_2 : 0.125). Overestimations of such values in normoxic conditions have previously been recorded (Brehm et al., 2003; Vogler et al., 2009). Variability in results at rest in normobaric hypoxia (FiO_2 : 0.125) by the MetaMax3X in comparison to the Douglas Bag method infers caution should be used in interpreting values obtained in such conditions as such discrepancies could introduce error into the accuracy of EE calculations, assessment of cardiovascular health or when assigning training loads based upon recorded metabolic values such as $\dot{V}O_2$. Resting measures in normobaric hypoxia were taken in order to simulate for conditions in which future clinical work will be conducted.

REPRODUCIBILITY

Acceptable reliability limits as suggested by Crouter et al. (2006) of <10% CVs for \dot{V}_E were recorded in the current study. Furthermore CVs of <10% were recorded for all four respiratory variables measured during repeated MetaMax3X trials. Reproducibility between duplicate MetaMax3X trials were similar for all measures (CV 5.4 – 6.9%). Reproducibility results were also similar to those found in previous studies conducted in normoxia (Crouter et al., 2006; Duffield et al., 2004). Lower variability in the current study was reported, in comparison to previous work (Crouter et al. 2006; Macfarlane and Wong 2012) for measures of $\dot{V}O_2$, $\dot{V}CO_2$ and \dot{V}_E . Alternatively, reproducibility measures in the current study compared to that of Vogler et al. (2010) display larger variability with a similar sized participant number for all measures with the exception of RER. Such variation in results across studies highlights the difficulty in drawing conclusions on acceptable limits of variability. Respiratory exchange ratio and \dot{V}_E also fall within the target typical error values suggested by the Australian Sports Commission at < 0.05 and < 5.0 L.min⁻¹ respectively. Variability as seen in typical errors, mean bias results and CV were deemed acceptable in the current study for repeated MetaMax3X trials in normobaric hypoxia (FiO_2 : 0.12) indicating that the MetaMax3X provides measures that are reproducible during rest in humans at normobaric hypoxia (FiO_2 : 0.12).

VALIDITY

Limits of agreement for measures of $\dot{V}O_2$ are lower in the current investigation compared to those reported, and deemed acceptable, in separate studies from Basset et al (2001) and McLaughlin et al. (2001) in which differing metabolic analysers were measured for accuracy against the criterion Douglas Bag. Values in the present investigation compared to the aforementioned results from Basset et al. (2001) -0.08 to 0.11 $L \cdot min^{-1}$ ($\dot{V}O_2$) Larsson et al. (2004) -0.33 to 0.15 $L \cdot min^{-1}$ ($\dot{V}O_2$) and Jakovljevic et al. (2008) -0.52 to 0.55 $L \cdot min^{-1}$ indicate, although significantly different, the current study has yielded smaller 95% confidence limits of agreement following comparison of the MetaMax3X with the criterion Douglas Bag in normobaric hypoxia FiO_2 : 0.12. Differences in accepted limits of agreement between various metabolic systems exist for the measurement of aerobic capacity, with such limits poorly defined in previous literature. In a study from Babineau et al., (1999) variances of 4% has been outlined as the limit of acceptability between measures of aerobic capacity for some national certification bodies which the current findings do not fall within. Some suggestions, however, widen these limits of acceptability with differences between 3% and 10% found in previous investigation (Hodges et al., 2005).

Previous studies assessing the validity of an identical system as the MetaMax3X, the Sensor Medics VmaxST, have produced varied results for validity measures with significantly higher values of measurement for values of EE and $\dot{V}O_2$ being reported compared to the Douglas Bag method (Brehm et al., 2004) yet these differences were described as small as differences were within 7.5% of Douglas bag values. The consensus was that data derived from the system were acceptable for determining EE at rest and at "walking pace", determined by Bland-Altman limits of agreement analysis, and equivalent to the Douglas bag method of analysis at rest and sub-maximal exercise (Brehm et al., 2004) and during sub-maximal exercise alone.

Discrepancies in $\dot{V}O_2$ and RER corresponding to typical errors of 0.03 $L \cdot min^{-1}$ and 0.05 respectively in the present study may result in differences of 19% in EE when comparing Douglas bag values of $\dot{V}O_2$ and RER with MetaMax3X values. Calculated EE values of 1.76 $Kcal \cdot min^{-1}$ from Douglas bag values were recorded compared to that of 2.17 $Kcal \cdot min^{-1}$ from the MetaMax3X equating to a difference of 25 $kcal \cdot hr^{-1}$ and 600 $kcal \cdot day^{-1}$. These findings result in meaningful differences when used to estimate expenditure over a prolonged period of time and thus cannot be accepted as valid when using these values to calculate EE. As such values obtained from the MetaMax3x in normobaric hypoxia should not be used in order to make suggestions relating to EE over an extended time period. Furthermore such discrepancies in $\dot{V}O_2$ and RER will also induce meaningful differences in substrate partitioning. Specifically an increase in fat use by as much as a $4.5g$ increase in fat utilisation and a $5.9g$ decrease in CHO utilisation could be reported over the 45 minute exposure when using values of RER and $\dot{V}O_2$ from MetaMax3X values compared to those obtained by Douglas Bag. Such changes in RER may

impact upon research in an exercise and health setting and resulting in important information being misinterpreted and miss-represented.

Overestimations of metabolic rate, when devices are compared to criterion methods, are seen consistently. Such overestimations compared to the Douglas bag, described from a number of studies including Macfarlane et al. (2012) in which % differences in mean $\dot{V}O_2$ between the MetaMax3B and the Douglas bag were reported at rest ($10.6 \pm 19.3\%$) and during both moderate ($9.7 \pm 13.2\%$) and vigorous exercise ($11.8 \pm 7.6\%$), are reflected in the present data. Further overestimations of $\dot{V}O_2$ values in differing metabolic analysers are also reported including the Medical Graphics system which $\dot{V}O_2$ values were greater by $0.268 \text{ L}\cdot\text{min}^{-1}$ (La Mere et al., 1993) and the SensorMedics by $0.208 \text{ L}\cdot\text{min}^{-1}$ during (Babineau et al., 1999).

Nevertheless the current study has reported significantly higher $\dot{V}O_2$ values (21 %) from the MetaMax3X when compared with the Douglas bag, as seen in previous investigations (Vogler et al., 2010). Discrepancies of 21% in $\dot{V}O_2$ values in this study are a cause for concern, resulting in an average $1.39 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ $\dot{V}O_2$ at rest greater from MetaMax3X sessions compared to Douglas bag measures. Possible explanations for such discrepancies may be due to the method and equations of the online analyser to estimate BTPS, as has been previously suggested (Hodges et al., 2005) which, depending on environmental temperature, could result in a correction factor as large as 10% and thus resulting in some error. This suggestion, however may not be applicable to current findings as the environmental conditions were well controlled for both ambient temperature and $\text{Fi}O_2$, furthermore the physical chemistry used for the conversion equation is well understood.

Crouter et al. (2006) suggested that the VO2000, an alternate online metabolic analyser, tends to underestimate $\dot{V}O_2$ and $\dot{V}CO_2$ at lighter work rates yet overestimate the same values at higher work rates. This point may therefore be applicable to the current investigation due to the well-known increase in \dot{V}_E rates following exposure to hypoxic conditions (Ainslie et al., 2013) thus resulting in higher resting values compared to sea level conditions. In comparison to % changes outlined by Crouter et al. (2006) the results from the current study have markedly smaller variation in values of $\dot{V}O_2$ (21%), $\dot{V}CO_2$ (10%) and \dot{V}_E (5%) being recorded respectively in contrast to 53%, 46% and 12% from Crouter et al. (2006) for the same values.

Differences in measures of $\dot{V}O_2$ recorded by the MetaMax3X compared to the Douglas Bag in the current study may be attributed to the method of data capturing (Brehm et al. (2003). When analysing differences between trials the inclusion or exclusion of one breath at a frequency of 12 breaths per minute could lead to an 8% difference in \dot{V}_E values. Crouter et al. (2006) highlighted the inclusion of resting data as a main limiting factor for increased variability in measures. The number of whole breaths as indicators of collection, as oppose to a time period, may provide lower errors and differences. Brehm et al. (2003) further found that, similarly to the current investigation, the SensorMedics VmaxST measured significantly higher $0.02 \text{ L}\cdot\text{min}^{-1}$ values than Douglas bag

measurements of oxygen uptake at rest. These findings echo earlier data presented by Webster et al. (1998), Medbo et al. (2002) and the aforementioned Vogler et al. (2009).

Work carried out on earlier versions of the MetaMax (versions I and II) reported different accepted limits of variance and therefore result in difficulty in drawing definite conclusions regarding the use of the MetaMax3X in normobaric hypoxia. Overall the MetaMax3X displayed sufficient indication of the physiological demands on both occasions albeit with slight variations between repeated trials. Variations compared to criterion measures highlight differences that will induce meaningful differences for both actual and derivative values specifically EE and highlights the need for rigorous testing of research equipment for use in specific environmental conditions.

LIMITATIONS AND FUTURE DIRECTIONS

Ideally simultaneous measures using the DB and MetaMax3X would be taken in order to prevent the day to day variation in measurement (Brehm et al., 2004), estimated to be about 3-4% for $\dot{V}O_2$ (Vogler et al., 2010, 2007). As this was not possible due to the nature of MetaMax3X collection in the normobaric hypoxic environment, reproducibility measures were taken on two separate occasions with validity being assessed on a third occasion through the criterion method with sessions being matched for time of day and environmental conditions. Furthermore, each session was separated by a week in order to avoid any acclimation response from the hypoxic exposure. Thus some of the described differences seen between methods may be attributable to the non-simultaneous measures and can be considered a limitation to the study. Repeated trials on human participants combine the error of both a biological and technical error, culminating in a greater variation of values (Macfarlane 2001). Nevertheless, protocols of a similar nature (Perkins et al. 2004) have been sufficient in providing results with satisfactory levels of agreement.

The assumption of protein oxidation remaining constant throughout may also be raised as a limitation whilst extrapolating information from RER values. Based on the higher oxygen cost of protein (Charlot et al., 2013) and fat it has been suggested that carbohydrate is the preferred fuel in hypoxia (McClelland, 2004). Nevertheless changes in protein oxidation due to hypoxic conditions may further reduce the strength of RER as a reliable measure in the health setting. Lower RER values from the MetaMax3X were reported throughout the exposure against the Douglas bag standard. Trends in the data suggest that the changes in this underestimation in RER are results of the changes in $\dot{V}CO_2$ (Figure 4.2, Graphs B and C). Seemingly a larger underestimation in RER corresponds with an overestimation of $\dot{V}CO_2$.

This study has brought forward previous investigations by conducting reproducibility and validity measures on an online metabolic analyser in an environment of normobaric hypoxia. Further analysis regarding the reproducibility and validity of the MetaMax3X should be conducted in differing levels of hypoxia. Future work should also focus on the reproducibility and validity of differing exercise

intensities in hypoxic conditions in order to have a complete picture of human performance within these environments. The effect of hypoxia on the metabolic markers measured by gas analysis should also be examined in order to further understand the acute effects of reduced oxygen availability on markers that may influence changes in body mass. This study sits within the overall aim of the thesis by providing validity and reproducibility data of the MetaMax3X which is used upon ascent to altitude in study 4 (Chapter 7).

4.6 CONCLUSION

These results indicate that the MetaMax3X has acceptable levels of reproducibility following repeated trials in normobaric hypoxia (F_{iO_2} : 0.12) that are comparable to previous investigations (Bassett et al., 2001). Overall the MetaMax3X displayed sufficient indication of the physiological demands on both occasions with repeated trials indicating good agreement for values of $\dot{V}O_2$, $\dot{V}CO_2$ RER and \dot{V}_E .

Validity measures indicated significant differences in values of and meaningful differences in subsequent calculations of EE and thus cannot be considered valid. Variability in measures at rest between the MetaMax3X and the criterion Douglas Bag suggests that there should be caution in interpreting the values obtained by the MetaMax3X in normobaric hypoxia and highlights potential problems in using the two systems interchangeably. Variations compared to criterion measures highlight the need for rigorous testing of research equipment for use in specific environmental conditions.

5. AN ACUTE EXPOSURE TO $\text{FiO}_2:0.12$ INDUCES ALTERATIONS IN MARKERS OF LIPID METABOLISM IN A HEALTHY HUMAN POPULATION

5.1 ABSTRACT

Aim: The purpose of this study was to determine the effects of an acute exposure to normobaric hypoxia ($\text{FiO}_2: 0.12$) at rest on; resting metabolic rate (RMR), substrate utilisation, measures of free fatty acids (FFA) and triglycerides (TAG) in human participants. **Method:** An experimental group consisting of ten healthy volunteers (2 female, 8 male) were exposed to hypoxia ($\text{FiO}_2: 0.12$) at rest for 45 minutes following a period of 30 minutes rest in normoxia. A control group consisting of a different set of ten healthy volunteers (5 female, 5 male) were exposed to normoxia ($\text{FiO}_2: 0.21$) and measured for 45 minutes. Measures included expired air; RMR by indirect calorimetry, venous plasma FFA and TAG recorded every 15 minutes. **Results:** There were no changes in $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, RER, \dot{V}_E , EE, FFA and TAG during a 45 minute rest period in the control group following a 12 hour fast. Measures of $\dot{V}\text{O}_2$ (13%), $\dot{V}\text{CO}_2$ (40%), \dot{V}_E (37%), HR (10%) and RER (25%) were all found to be elevated ($p < 0.05$) in hypoxia compared to pre-measures in the experimental group at $\text{FiO}_2: 0.12$. Acute hypoxia increased venous FFA from $0.38 \pm 0.14 \text{ mmol.L}^{-1}$ pre-exposure to $0.56 \pm 0.18 \text{ mmol.L}^{-1}$ post-exposure ($p < 0.05$) and increased TAG from $207.36 \pm 83.87 \text{ mg.dL}^{-1}$ pre-exposure to $261.94 \pm 146.67 \text{ mg.dL}^{-1}$ post-exposure ($p < 0.05$) in the experimental group. There was an increase in RMR with exposure to hypoxia, from 5802 J.min^{-1} ($1.4 \text{ kcal.min}^{-1}$) to 7776 J.min^{-1} ($1.9 \text{ kcal.min}^{-1}$) ($p < 0.001$). Acute hypoxia induced an increase in metabolic rate, due to increases in the contribution of both carbohydrate (CHO) and fat utilisation to EE compared to pre-measures. **Conclusions:** An acute bout of hypoxia appeared to be sufficient to induce rapid alterations in substrate utilisation and blood measures of lipid metabolism, illustrated through changes in levels of FFA, TAG and RMR. If sustained this would lead to increased EE, at rest, of 2000 J.min^{-1} ($0.5 \text{ kcal.min}^{-1}$) above that of normoxia.

5.2 INTRODUCTION

Exposure to reduced oxygen availability has the potential to elicit beneficial effects on cardiovascular health (Schobersberger et al., 2003). Increased EE, and the subsequent breakdown of energy stores such as fat, has been shown to result from time spent at altitude amongst both healthy (Westerterp et al., 2000) and obese individuals. Furthermore chronic exposure to hypoxia has shown reduced adiposity after five days (Lippl et al., 2010). Observed decreased arterial blood pressure (Lippl et al., 2010), increased peripheral vasodilation and an improved insulin sensitivity (Schobersberger et al., 2003) support the suggestion that hypoxic exposure of an acute (Reynolds et al., 1999), chronic (Lippl et al. 2010; Schobersberger et al. 2003; Westerterp and Kayser 2006) or intermittent (Wiesner et al., 2010) nature may be a worthwhile method for inducing various health benefits.

Fat is stored as triacylglycerol (TAG) in fat cells at various sites around the body. Triglycerides may be broken down in response to sympathetic stimulation via the activation of hormone sensitive lipase (HSL) to release glycerol and free fatty acids (FFA). Free fatty acids represents an important energy source for the body and is the primary oxidative fuel for resting skeletal muscle (Boden and Shulman, 2002). Availability of FFA is increased following the stimulation of adipose tissue lipolysis (catabolism of triglycerides to FFA and glycerol molecules) when demand for fuel rises, such as during conditions of starvation and exercise.

Oxygen supply is known to be one of the environmental conditions that have a stimulating effect on resting energy expenditure (EE) (Oltmanns et al., 2006). Exposure to high and extreme altitudes (5,300m – 8,848m) where hypoxia and reduced blood oxygen saturation occurs, have been demonstrated to elicit a steady reduction in weight proportional to increased severity of hypoxia (Reynolds et al., 1999). Although the exact mechanisms behind such findings are unclear a negative energy balance due to increased EE and reduced energy intake are often the main contributors ascribed to body mass loss at altitude (Kayser and Verges, 2013; Lippl et al., 2010; Mawson et al., 2000; Westerterp, 2001; Westerterp-Plantenga et al., 2011) with such losses in body mass being predominantly recorded as reductions in fat mass (Chen et al., 2010; Lippl et al., 2010; Reynolds et al., 1999).

Rises in EE occur due to an increased basal metabolic rate and increased physical activity during high altitude sojourns (Macdonald et al., 2009). Suppression of appetite, reduced availability of food and a subsequent reduction in food intake have also been indicated as reasons for changes in body mass at altitude, as have carbohydrate mal-absorption and changes in fat metabolism (Hamad and Travis 2006). Hypoxia alone, and when coupled with exercise, has been shown to raise lipid oxidation (Wiesner et al., 2009) and to induce an increase in the production of the hypoxia-induced factor-alpha 1 (Netzer et al., 2007) which has been linked to changes in food intake and increases in EE following alterations in cellular metabolism, activation of the central nervous system and peripheral pathways (Palmer and Clegg 2014).

Lasting increases in the EE of participants, changes in body composition and induced weight loss (Tanner et al., 1998) indicate the potential for the use of exposure to hypoxia as a fat and weight loss method or to manage body composition. The effects of acute exposure to severe hypoxia on such parameters however is not well established, while no previous studies have investigated the effects of a single, acute bout of normobaric hypoxia on FFA, TAG in human participants. Lengthy exposures to altitude are not a feasible option for many individuals aiming to lose weight. A greater understanding of the underpinning mechanisms leading to weight loss, particularly through increased EE and alterations in metabolism, may lead to the development of new, effective and more feasible strategies of weight loss in humans. Therefore the primary aim of the current study were to 1) Determine the effects of an acute bout of normobaric hypoxia at rest on substrate partitioning, EE, and circulating FFA, TAG in healthy individuals. It was hypothesised that hypoxia will acutely induce cardiovascular and respiratory responses and increase resting metabolic rate fuelled predominantly by an increase in CHO partitioning. This present study sits within the overall aim of the thesis by providing information regarding an acute, severe bout of hypoxia which will be viewed in the context of a more prolonged exposure and used in combination with an exercise trial in study 3(Chapter 6).

5.3 MATERIALS AND METHODS

PARTICIPANTS

Twenty physically active individuals agreed to participate in the study. Ten of the individuals (8 males, 2 females) 24 ± 4 years of age (mean \pm SD), body mass 77 ± 10 kg, height 178 ± 9 cm and BMI 24 ± 2 kg/m² formed an experimental group. The remaining 10 participants (n = 10: 5 female, 5 male) 22 ± 3 years of age, body mass 72 ± 11 kg, height 175 ± 10 cm and BMI 23 ± 1 formed a control group. A control group was used in order to record the effects of a similar period of fasting in a similar healthy population following a 12 hour fast.

All participants complied with all criteria for participation [(3.5.1) Medical criteria and recruitment and (3.6) Pre-trial diet and exercise standardisation and (3.8) Hydration assessment]. Prior to the undertaking of the experimental trials volunteers attended the laboratories whereby their anthropometric data was collected [(3.11) Anthropometric assessment]. Female participants were tested throughout the menstrual cycle thus the use of contraceptive medication is likely.

EXPERIMENTAL DESIGN

The study required each group of participants to attend the laboratory on one occasion. Participants in the experimental group were exposed to FiO₂: 0.12 at rest in a purpose built nitrogen enriched hypoxic chamber (The Altitude Centre, UK) for a period of 45 minutes (HYP) following a period of 30 minutes rest in normoxia (FiO₂: 0.21) (PRE). The chamber environment was controlled for each trial [(3.12.2) Controlling the hypoxic environment]. Participants in the control group were monitored for

45 minutes of sitting at FiO_2 : 0.21, following a 30 minute rest period at FiO_2 : 0.21 [(3.12.1) Ambient laboratory temperature control].

BLOOD SAMPLING AND ANALYSIS METHODS

Venous blood samples were taken [(3.17.1) Cannulation] pre and post-trial and at 30 minutes during the trial. With plasma separated [(3.17.2) Blood plasma separation] FFA and TAG were analysed [(3.17.3) Analysis of FFA and (3.17.4) Analysis of TAG].

GAS MEASURES AND PHYSIOLOGICAL DATA

Expired gas was collected and analysed using the Douglas bag method [(3.13.1) Douglas bag metabolic gas analysis]. Resting metabolic rate and substrate utilisation were determined indirectly via non-protein respiratory exchange ratio which was determined through expired gas. Non-protein RER assumes constant protein oxidation. Oxidation rates of fat and carbohydrate (CHO) were calculated according to the following equations outlined (Peronnet and Massicotte 1991) (3.13.3) Calculations derived from cardiopulmonary measures). Total energy expenditure (TEE) was calculated by assuming constant expenditure at each time point for 5 min. Heart rate and SpO_2 were measured at each 5 minute interval during exposure [(3.13.5) Heart rate and (3.13.4) Peripheral artery oxygen saturation].

MONITORING FOR SYMPTOMS OF ACUTE MOUNTAIN SICKNESS (AMS)

During exposure to FiO_2 : 0.12 a modified Lake Louise Questionnaire (LLQ) (Roach et al., 1993) was used to monitor symptoms of AMS at every 10 minute interval [(3.14.1) Lake Louis Questionnaire score].

STATISTICAL ANALYSIS

All data were checked for normality and sphericity [(3.15.2.1) Normal distribution and (3.15.2.2) Sphericity]. All data were analysed using a standard statistical package (SPSS version 20 for Windows 7). One way within subject analysis of variance with repeated measures (ANOVA) was used to compare for differences in all variables throughout the 45 minute exposure to normoxia and hypoxia (between groups). A post-hoc Bonferoni pairwise analysis was carried out to determine at which time points differences were seen. Paired sample T-tests were used to examine the differences between normoxic and hypoxic physiological measures. Data are reported as mean \pm SD, with the significance level set at $p \leq 0.05$.

5.4 RESULTS

CONTROL GROUP

There was no changes in measures of $\dot{V}O_2$ (0.26 ± 0.04 L.min⁻¹) ($F_{(8, 64)} = 1.273, p = 0.274, np^2 = 0.137$), $\dot{V}CO_2$ (0.21 ± 0.03 L.min⁻¹) ($F_{(8, 64)} = .273, p = 0.973, np^2 = 0.033$), RER (0.79 ± 0.1) ($F_{(8, 64)} = .691, p = 0.698, np^2 = 0.080$), \dot{V}_E (3.2 ± 1.7 L.min⁻¹) ($F_{(8, 64)} = 2.072, p = 0.110, np^2 = 0.206$), HR (59 ± 11 b.min⁻¹) ($F_{(8, 64)} = 1.502, p = 0.174, np^2 = 0.158$), SpO_2 (99 ± 1) ($F_{(8, 64)} = .645, p = 0.737, np^2 = 0.075$) and EE (3.27 ± 0.83 kJ) ($F_{(8, 64)} = 1.180, p = 0.325, np^2 = 0.129$) throughout a 45 minute rest period in $FiO_2:0.21$ in the control group. No change was observed in CHO (69 ± 28 %) ($F_{(8, 64)} = .706, p = 0.685, np^2 = 0.081$) or fat (31 ± 28 %) ($F_{(8, 64)} = .705, p = 0.686, np^2 = 0.081$) utilisation throughout the control group trial. Similarly no changes in FFA ($F_{(2, 12)} = 2.174, p = 0.156, np^2 = 0.266$) and TAG ($F_{(2, 12)} = 2.659, p = 0.356, np^2 = 0.458$) were induced through a 45 minute rest period at $FiO_2: 0.21$ as shown in Table 5.1.

EXPERIMENTAL GROUP

Exposure to $FiO_2: 0.12$ elicited elevation of $\dot{V}O_2$ (PRE 0.29 ± 0.02 HYP 0.35 ± 0.03), ($t_{(9)} = -4.941, p = 0.001, d = 2$), $\dot{V}CO_2$ (PRE 0.24 ± 0.04 HYP 0.33 ± 0.04) ($t_{(9)} = -6.262, p < 0.001, d = 2.75$), RER (PRE 0.82 ± 0.17 HYP 0.95 ± 0.13) ($t_{(9)} = -2.791, p = 0.021, d = 1.31$), \dot{V}_E (PRE $5. \pm 2.8$ HYP 11.6 ± 2.3), ($t_{(9)} = -5.443, p < 0.001, d = 2.96$) and EE (PRE 5.8 ± 0.4 HYP 7.3 ± 0.4 kJ) ($t_{(9)} = -8.588, p < 0.001, d = 2.59$) compared with pre-values obtained in normoxia. An increase in HR (PRE 61 ± 16 HYP 66 ± 13) ($t_{(9)} = -2.737, p = 0.023, d = 0.34$), and a decrease in SpO_2 (PRE 98 ± 1 HYP 84 ± 4) ($t_{(9)} = 11.166, p < 0.001, d = 5.05$) when compared with pre-values in normoxia were induced at $FiO_2: 0.12$ in the experimental group at the same time point.

Measures of FFA ($F_{(2, 54)} = 7.908, p = 0.001, np^2 = 0.227$) and TAG ($F_{(2, 54)} = 4.388, p = 0.032, np^2 = 0.128$) were different over time. An acute 45 minute exposure to $FiO_2: 0.12$ induced an increase in circulating FFA ($p = 0.001, d = 0.96$) and TAG ($p = 0.04, d = 0.50$) resulting in a 54% and a 26% increase respectively from pre-to post-exposure in the experimental group. There was no increase in FFA within the first 30 minutes of hypoxia as shown in Table 5.1. Triglycerides were increased at 30 minutes compared to pre-values ($p = 0.03, d = 0.37$). Increases of 12% and 10% were recorded post-exposure when compared to values at 30 minutes for FFAs ($p = 0.316, d = 0.38$) and TAGs ($p = 0.739, d = 0.23$) respectively although not significant.

Throughout exposure to the 45 minute hypoxic environment differences were recorded for $\dot{V}O_2$, ($F_{(8, 72)} = 8.138, p = 0.001, np^2 = 0.475$), RER ($F_{(8, 72)} = 2.869, p = 0.036, np^2 = 0.242$) and EE ($F_{(8, 72)} = 8.481, p < 0.001, np^2 = 0.485$). No changes were found for $\dot{V}CO_2$ ($F_{(8, 72)} = .708, p = 0.683, np^2 = 0.073$), \dot{V}_E ($F_{(8, 72)} = .498, p = 0.702, np^2 = 0.052$) or HR ($F_{(8, 72)} = 1.095, p = 0.377, np^2 = 0.108$) throughout the 45 minutes. Respiratory exchange ratio values were at their highest and elevated at the 15 minute time

point, corresponding with the lowest value of $\dot{V}O_2$. EE was altered throughout the 45 minute exposure and peaked on 45 minutes at $7.78\text{kJ}\cdot\text{min}^{-1}$ ($1.9\text{ kcal}\cdot\text{min}^{-1}$). $\dot{V}O_2$ values also peaked at 45 minutes ($0.38\text{ L}\cdot\text{min}^{-1}$). A 25% mean increase in EE was recorded from pre-measures to hypoxic exposure. All physiological values taken throughout exposure to hypoxia are shown in Table 5.2.

Differences were recorded for CHO utilisation expressed as $\text{g}\cdot\text{min}^{-1}$ throughout exposure to normobaric hypoxia ($F_{(9, 81)} = 3.533$, $p = 0.001$, $np^2 = 0.282$). Values of $0.7 \pm 0.11\text{ g}\cdot\text{min}^{-1}$ were recorded at minute 45 highlighting increases compared to values of $0.45 \pm 0.09\text{ g}\cdot\text{min}^{-1}$ at minute 5 ($p = 0.004$, $d = 2.44$). Fat utilisation also differed throughout exposure to reduced oxygen availability ($F_{(9, 81)} = 2.821$, $p = 0.036$, $np^2 = 0.239$) highlighting an increase at minute 45 when individuals were utilising $0.1 \pm 0.05\text{ g}\cdot\text{min}^{-1}$ compared to minute 5 when individuals were utilising $0.04 \pm 0.02\text{ g}\cdot\text{min}^{-1}$ ($p = 0.038$, $d = 1.46$). Increases in the contribution of CHO to EE throughout exposure increased from $0.87\text{ kcal}\cdot\text{min}^{-1}$ prior to exposure to a mean value of $1.31 \pm 0.14\text{ kcal}\cdot\text{min}^{-1}$ (+ 51%) and a peak value of $1.47\text{ kcal}\cdot\text{min}^{-1}$ throughout exposure. Increased contributions from fat sources were seen from a pre-value contribution of $0.52\text{ kcal}\cdot\text{min}^{-1}$ to a peak value of $0.67\text{ kcal}\cdot\text{min}^{-1}$ (+ 31%) throughout exposure. Differences in TEE were recorded throughout the trial ($F_{(9, 81)} = 20.308$, $p < 0.001$, $np^2 = 0.693$) with increases in values observed at each time point throughout exposure to hypoxia when compared to pre-measures (< 0.05).

TABLE 5.1: Mean \pm SD data for pre, 30 minute, and post-values for blood markers in the control and hypoxic group exposed to [Fraction of inspired Oxygen FiO_2 : 0.21].

Control Group

	Pre	30 minutes	Post
FFA ($\text{mmol}\cdot\text{L}^{-1}$)	0.33 ± 0.09	0.30 ± 0.13	0.26 ± 0.09
TAG ($\text{mg}\cdot\text{dL}^{-1}$)	215.65 ± 54.25	203.68 ± 87.46	209.56 ± 63.52

Hypoxic Group

	Pre	30 minutes	Post
FFA ($\text{mmol}\cdot\text{L}^{-1}$)	0.38 ± 0.14	0.44 ± 0.15	$0.56 \pm 0.18^*$
TAG ($\text{mg}\cdot\text{dL}^{-1}$)	207.36 ± 83.87	$245.6 \pm 99.9^*$	$261.94 \pm 146.67^*$

Notes: * Denotes significant difference when compared with pre-values within groups. Free fatty acids (FFA), triglycerides (TAG). All significance symbols correspond to $p < 0.05$.

TABLE 5.2: Mean \pm SD data for physiological measures throughout the exposure to hypoxia in the hypoxic group.

	Time (minutes)								
	5	10	15	20	25	30	35	40	45
$\dot{V}O_2$ (L.min ⁻¹)	0.33 \pm 0.02	0.36 \pm 0.01	0.31 \pm 0.02	0.36 \pm 0.03	0.35 \pm 0.04	0.37 \pm 0.03	0.36 \pm 0.03	0.35 \pm 0.04	0.38 \pm 0.01*
$\dot{V}CO_2$ (L.min ⁻¹)	0.33 \pm 0.02	0.34 \pm 0.01	0.33 \pm 0.02	0.34 \pm 0.03	0.33 \pm 0.04	0.33 \pm 0.03	0.32 \pm 0.03	0.35 \pm 0.04	0.32 \pm 0.01
\dot{V}_E (L.min ⁻¹)	12.1 \pm 1.9	11.4 \pm 1.8	11.2 \pm 1.2	11.6 \pm 1.5	11.9 \pm 2.4	11.3 \pm 1.8	11.7 \pm 2.6	12.4 \pm 3.4	11. \pm 3.4
RER	0.99 \pm 0.07	0.95 \pm 0.15	1.05 \pm 0.13*	0.97 \pm 0.20	0.95 \pm 0.12	0.90 \pm 0.1	0.90 \pm 0.09	0.99 \pm 0.13	0.85 \pm 0.08
EE (kcal.min ⁻¹)	1.65 \pm 0.09	1.75 \pm 0.05	1.56 \pm 0.08	1.75 \pm 0.11	1.75 \pm 0.11	1.84 \pm 0.12	1.77 \pm 0.11	1.75 \pm 0.17	1.86 \pm 0.04*
CHO (%)	89 \pm 14	69 \pm 25	89 \pm 22	69 \pm 32	76 \pm 32	63 \pm 29	64 \pm 26	83 \pm 25	79 \pm 34*
FAT (%)	11 \pm 14	31 \pm 25	11 \pm 22	31 \pm 32	24 \pm 32	37 \pm 29	36 \pm 26	17 \pm 25	21 \pm 34*
HR (b.min ⁻¹)	67 \pm 16	67 \pm 15	68 \pm 15	65 \pm 13	65 \pm 12	65 \pm 14	64 \pm 13	66 \pm 13	64 \pm 13
SpO ₂ (%)	85 \pm 3	85 \pm 3	83 \pm 3	82 \pm 3	83 \pm 4	83 \pm 3	84 \pm 4	83 \pm 3	84 \pm 7

Notes: Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation (\dot{V}_E) and respiratory exchange ratio (RER), energy expenditure (EE), carbohydrate utilisation (CHO), fat utilisation (FAT), heart rate (HR) and peripheral oxygen saturation (SpO₂). * represents significant difference compared to 5 minute values. All significance symbols correspond to $p < 0.05$.

5.5 DISCUSSION

The development of normobaric and hypobaric chambers in recent years has meant that exposure to hypoxia can now more readily occur for acute periods in a controlled setting. With this in mind the current study investigated the effects of an acute bout of normobaric hypoxia (FiO_2 : 0.12) on substrate utilisation and blood measures of metabolism, illustrated through levels of FFA and TAG, in a population of healthy participants with normal BMI's.

BLOOD LIPID RESPONSES

Considerable weight loss observed in lowlanders at high altitude has been described as an inevitable consequence to chronic hypobaric hypoxia (Kayser, 1992). As such the use of hypoxia as a potential mechanism for reductions in body mass for unhealthy individuals has been suggested (Lippl et al., 2010). Chronic exposure to hypoxia/ altitude however is not often practical or available for the lowlander. The use of acute bouts of hypoxia, either single or repeated, may offer a therapeutic effect on physiological variables that are contributory to reductions in body mass. A major part of the reported losses in body mass have been attributed to reductions in fat mass (Reynolds et al., 1999) leading to the suggestion exposure to hypoxia can induce increases in lipid metabolism in humans. Significant increases in resting FFA and TAG values were found in the current study following a 45 minute exposure to normobaric hypoxia (FiO_2 : 0.12) corresponding to percentage increases of 54 and 26% respectively. This is in comparison to no significant changes being recorded in a healthy control group for the same measures following a 45 minute rest period at FiO_2 : 0.21. Current findings indicate that a 45 minute, resting exposure to normobaric hypoxia (FiO_2 : 0.12) was sufficient to infer an increase in EE and an alteration in lipid metabolism in a group of healthy human participants displayed by increases in circulating FFA and TAG.

In order to be oxidised, lipid stores within the body must first be mobilised from triacylglycerides into FFA (McClelland, 2004). Mammalian plasma FFA have previously been reported to be elevated when measured at altitude following chronic exposure, attributed to an increased reliance on lipid metabolism at altitude to conserve valuable carbohydrate stores following acclimation (Young et al., 1982 in McClelland, 2004). There is, however, a lack of agreement concerning the effects of altitude on substrate metabolism with increases in carbohydrate utilisation at altitude more frequently recorded (Brooks et al., 1991; Braum et al., 2000; Roberts et al., 1996). Such conclusions have been attributed to the "oxygen saving effect" of carbohydrate utilisation suggested by previous research based on fewer moles of oxygen required to obtain energy from glucose compared to obtaining energy from fat whilst in conditions where oxygen supplies are limited.

Drawing conclusions on the hypoxic induced changes in lipid metabolism is therefore challenging from this previous equivocal data and is further confounded by differences in the duration and severity of exposure amongst previous research (Kennedy et al., 2001). Furthermore few studies investigating the effects of an acute bout of hypoxia and no previous research, to the author's knowledge, investigating parameters of metabolism in a human population have been conducted.

High altitude acclimatisation results have indicated a sparing of muscle glycogen stores through a greater rate of fat utilisation are based upon elevated levels of FFA in the blood previously seen in both rats (Yin et al., 2008; Larsen et al., 1989) and man (Jones et al., 1972), along with increased glycerol concentrations and a slower rate of muscle glycogen utilisation. Although significant increases in FFA have been reported in the current study such findings do not necessarily translate to an increased rate of fat oxidation as a substrate (Kennedy et al., 2000) as highlighted by a greater proportional increase in CHO utilisation in the present study and a previously stated simultaneous hypoxia induced increase in lipolysis and reduction of FFA uptake (Benso et al., 2007; Yin et al., 2008). These findings may perhaps be explained by the fact that the mobilisation and transport of fatty acids is a slow process and at rest 70% of FFA released are recycled back to TAG via intracellular and extracellular routes (McClelland, 2004). The length of exposure rather than the severity of hypoxia in the present study may therefore have been a contributing factor in the lack of change in utilisation of FFA as a substrate. This is despite the exposure being sufficient to increase EE and a greater release of FFA and TAG

Similarly to current findings short term exposure to sustained hypoxia has induced hypertriglyceridemia in humans (Barnholt et al., 2006; Farias et al., 2006). It has also previously been reported, following chronic intermittent exposure to graded hypoxia (FiO_2 : 0.21, 0.17, 0.14, 0.10, 0.07), increases in both plasma and liver TAG levels which were correlated with increasing severities of FiO_2 in rats (Jun et al., 2012). Interestingly it has been hypothesised that these increases in recorded values may be due to an increased hepatic secretion and decreased lipoprotein clearance (Jun et al., 2012). In an attempt to account for the effects of TAG changes over time, levels of TAG were assessed at 2 hour intervals during exposure to both FiO_2 : 0.21 and 0.10. As expected, TAG levels reduced as the length of time in a fasted state increased. Crucially however this was seen to occur more slowly in mice exposed to hypoxia and thus it was concluded that exposure to acute hypoxia rapidly delays postprandial TAG clearance with this effect then normalised as oxygen levels are restored (Jun et al., 2012). This effect was attributed to a reduction in brown and white adipose tissue lipoprotein lipase activity and was seen in conjunction with a decreased uptake and oxidation of FFA. Present results are further supported in a study from Roberts et al., (2012) who, following acute exposure (< 4 hours) to 4,300m recorded a significant decrease in the uptake of FFA and in glycerol release by resting muscle despite increases in plasma FFA being found in human participants. These findings were reported in conjunction with an increase in blood glucose dependence as indicated in the present study through an increased dependency on CHO during exposure.

In further support is work from Kennedy et al. (2012) in which down regulation of enzymes associated with fat metabolism were reported following both acute (24 hours) and chronic (two weeks) exposure to altitude recorded in rats. The activity of the down-regulated enzyme CPT-I is generally accepted to be largely responsible for regulating fatty acid oxidation coupled with the transport of long chain fatty acyl groups into the mitochondria. A down-regulation in this enzyme although not measured in the current study may provide a possible explanation for the findings. An inhibition of long-chain FFA entry into the mitochondria or into the muscle fibre (McClelland, 2004) and an inhibition of fatty acid transporter proteins FATP1 and CD36 by hypoxia (Yin et al., 2008) have also been suggested as possible mechanistic reasons for a reduction in FFA oxidation which may in part explain a reduction in fat utilisation at hypoxia.

RESTING METABOLIC RATE

Resting energy expenditure (REE) has been highlighted as the largest component of total daily energy expenditure, accounting for 60 to 75% of total expenditure (Melzer et al., 2007) highlighting it's importance for possible weight loss strategies. Increases in metabolic rate resulting in a discrepancy between energy intake and expenditure are previously demonstrated at altitude (Butterfield et al., 1992; Kayser, 1992; Lippl et al., 2010; Mawson et al., 2000). In concordance with previous research (Workman and Bassett, 2012), the present study has also demonstrated elevations in EE during acute hypoxia. Recent work from Workman and Bassett, (2012) recorded increases in EE of 16% following a 3 hour exposure to moderate hypoxia. Increases in EE in the current study of 25% are similar albeit slightly increased compared with this finding and other previous findings (Lippl et al., 2009) indicating the effect of hypoxia on EE independent of confounding factors normally associated with altitude exposure. Similar findings from previous research in which elevations in basal metabolic rate (BMR) occur in a dose dependant relationship with increases of 27% being reported following two days at 4,300m (Butterfield et al., 1992).

Mechanisms underpinning increased metabolic rate at altitude, although widely discussed, are not yet fully understood (Palmer and Clegg 2014; Westerterp 2001). Suggested reasons for this accepted outcome at altitude, aside from confounding factors associated with altitude such as cold exertion, include an increase in sympathetic drive (Mawson et al., 2000) and an induced higher basal noradrenalin levels (Urdampilleta et al., 2012) which may in part explain present findings. Such increases in metabolic rate are commonly associated with losses in body mass and thus support the suggestion that hypoxic stimulus can be effective as an approach to induce a novel, therapeutic tool for weight loss.

SUBSTRATE UTILISATION

Following exposure to altitude substrate partitioning has been altered (Workman and Bassett, 2012). As previously stated differences in experimental designs in relation to length and extremities of the exposure make it difficult to draw definitive conclusions and thus responses to hypoxia are still debated. Greater increases in both the oxidation of lipid and glucose sources have been demonstrated following exposure. Interestingly, studies in which increases in the oxidation of lipids are predominant (Louis et al, 2009; O'Donnell et al., 2007; Polotsky et al., 2003) have employed severe levels of hypoxia in their design (< 10% O₂) or use a repeated intermittent exposure protocol resulting in reduced glucose uptake or diminished glucose availability with subsequent increases in fat oxidation occurring as a secondary outcome due to a cumulative effect of repeated exposures (Workman and Bassett, 2012). Highlighted mechanisms for this change might occur through alteration of the neuroendocrine system. Alternatively a recent review from Palmer and Clegg (2014) investigating the effects of altitude for weight loss highlighted the hypoxic inducible factor HIF as an important mediator in the alterations in metabolism and EE induced by altitude. The review states that an up-regulation of the transcription factor HIF leads to a greater dependency on glucose uptake as an adaptive response to altitude when oxygen availability is limited which itself is linked to increased metabolic rate. The shift in utilisation as to increase dependence on glucose occurs in order to generate sufficient ATP whilst at altitude as the metabolism of glucose requires less oxygen compared to oxidative phosphorylation. In spite of this, the shift has been described as energy inefficient and the energy wasting effect of the shift has been highlighted as to play a role in the increase in metabolic rate.

In line with such suggestions, current findings display increased proportional contribution from CHO during exposure to an acute bout of hypoxia in combination with increased contributions of fat culminating in an increased energy expended. Increases in both fat and CHO contribution highlight a metabolic perturbation from acute hypoxia as suggested by Workman and Bassett (2012). Although both fat and CHO contribution to energy expended were seen to increase a greater proportional increase of 51% was seen in CHO. Such findings are supported by increased RER in the present study and consistent observations of increased utilisation of glucose, glycogen and lactate upon acute exposure in previous work (Brooks et al. 1991). Further examination of acute hypoxia on substrate utilisation is presented in study 3 (Chapter 6) of this thesis. A potential contributor to the magnitude of changes in substrate oxidation in the present study is the presence of both males and females in the participant group. A shift toward increased fat and blunted CHO use is observed in females upon exposure to 4,300m both acutely and for 10 days (Braun et al. 2000) potentially due to the presence of estrogen and progesterone (Bunt 1990). These findings suggest that it may be inappropriate to generalise findings from one sex to the other and that a conservation of glycogen stores at altitude is prioritised in females (Braun et al., 2000; McClelland et al., 1998).

LIMITATIONS AND FUTURE DIRECTIONS

Current findings have highlighted the effects of a single bout of an acute exposure to normobaric hypoxia on EE and circulating FFA and TAG as an indicator of metabolism. These observations should be further investigated in future studies for verification and in order to improve understanding of mechanistic factors leading to such changes in metabolism induced from hypoxic exposures. The data indicates that future investigations should also focus on hypoxia coupled with exercise as an attempt to harness the hypoxic induced increases in FFA recorded. Furthermore shifts in substrate partitioning and mechanisms underpinning such shifts may assist in the development of weight loss strategies for an overweight or obese population. As such, the following experimental chapter (Chapter 6) will investigate the combined effects of a single, acute passive exposure combined with a subsequent moderate intensity exercise bout on metabolic parameters similar to those in this study.

Limitations to the current findings however are present and results must be viewed in light of these. Of particular note is that direct comparisons cannot necessarily be drawn from a population with normal BMIs to an overweight or obese group (Lippl et al., 2010). The recruitment of both males and females and the lack of control for the menstrual cycle in females must also be viewed as a potential source of increased variation within the results and thus as a limitation to the investigation. Differences in metabolic rate throughout the menstrual cycle are observed (Solomon et al. 1982) and may therefore have contributed to the observed changes in the present study. Differences in the response to altitude with regard to substrate use is also seen amongst females compared to males (Braun et al. 2000; McClelland et al. 1998). Current findings must be viewed in light of this. This is of importance if future work focuses on the use of hypoxia as a tool for weight loss. Furthermore the studies measure only go some way in describing a physiological process rather than the underpinning cause and thus only suggestions based on previous literature are made as to the reasons for increases in EE, FFA and TAG. Separate experimental groups for each condition and limited participant number may also be considered an influencing limitation to the described work. Nevertheless current findings add to previous investigations and are novel in light of such findings occurring from an acute exposure of this length.

5.6 CONCLUSION

A single, acute exposure to normobaric hypoxia (FiO_2 : 0.12) lasting 45 minutes has inferred an increase in lipolysis demonstrated by increased circulating FFA and TAG in human participants. Increased RMR and changes in substrate utilisation were also recorded in the present study culminating in a greater reliance of carbohydrate utilisation throughout exposure, despite the aforementioned inferred lipolysis. Such findings indicate that single exposures lasting short time periods are able to induce changes in human participants that may be beneficial for the development of weight loss strategies in

humans; namely increases in EE and increased lipolysis in a thermo-neutral environment. These findings are of importance in light of the development of normobaric and hypobaric chambers meaning that exposure to hypoxic conditions can now more readily occur for acute periods in a controlled and safe setting.

THE SEPARATE AND COMBINED EFFECTS OF ACUTE HYPOXIA, MODERATE INTENSITY EXERCISE AND LIPID INGESTION ON MARKERS OF METABOLISM

6.1 ABSTRACT

Aims: 1. Assess the effects of acute hypoxia and a high fat meal (HLD) (883 kcal, 73g fat) on fuel use and resting metabolic rate (RMR). 2. Determine the effects of subsequent exercise on metabolic substrate use and supply. 3. Measure the effects of hypoxia, a HLD and exercise on Meteorin-like (Metrl) concentration. We hypothesised 1) Hypoxia would increase RMR and fat oxidation during exercise. 2) A HLD would increase fat oxidation. 3) Hypoxia and exercise may synergistically promote Metrl release. **Method:** Eight males (age, 22 ± 5 yr.; BMI 24 ± 4 kg/m²) completed five trials. One trial (CON) required participants to ingest a high lipid meal prior to a resting period of 240 minutes. Two trials required participants to ingest a high lipid meal prior to a 60 minute resting period in either FiO₂:0.12 (HYP) or FiO₂:0.21 (NORM) before completing 60 minutes of cycling exercise at 60% HRR in FiO₂:0.21. Two trials required participants to rest in either FiO₂:0.12 (HYPCON) or FiO₂:0.21 (NORMCON) before completing 60 minutes of cycling exercise at 60% HRR in FiO₂:0.21 without any prior ingestion of a high lipid meal. All trials were completed at 19°C, RH40%. **Results:** 1) HYP increased EE ($+0.42 \pm 0.18$ kcal.min⁻¹, +49%), carbohydrate (CHO) ($+0.23 \pm 0.22$ g.min⁻¹, +37%, $p=0.046$) and FAT oxidation ($+0.05 \pm 0.06$ g.min⁻¹, +12%, $p=0.003$) at 60min compared to NORM. 2) EE and fuel oxidation were unaltered ($p>0.05$) during exercise. Triglyceride concentration was lower ($-37 \pm 17\%$) when exercise occurred after a HLD compared to rest after a HLD. 3) Metrl was unaltered between conditions ($p=0.82$). **Conclusion:** Hypoxia increased EE through greater CHO and FAT use. Exercise reduced postprandial hypertriglyceridaemia. Hypoxia, exercise and a HLD are unimportant for acutely regulating Metrl concentration in humans.

6.2 INTRODUCTION

In 2014 more than 1.9 billion adults were overweight of which 600 million were considered to be obese (WHO, 2014). Excess fat mass is associated with increased risk of type 2 diabetes, hypertension, heart disease and some forms of cancer (Atkinson, 2014). A loss of fat mass and the redistribution towards greater lean mass is likely to be beneficial in relation to mortality and morbidity in individuals who are obese or overweight (Wirth et al. 2014). Chronic exposure to altitude induces increases in metabolism and substrate oxidation (Brooks, 2014; Brooks et al., 1991; Marriott and Carlson, 1996) highlighting hypoxia as a potential weight loss intervention.

During prolonged stays at altitude body mass decreases with a large proportion of loss attributable to fat (Lippel et al. 2010; Kayser and Verges 2013). Probable mechanisms underlying the observed changes in body mass and composition include increased metabolic rate (Brooks, 2014; Westerterp, 2001), appetite suppression (Lippel et al. 2010) and alterations in substrate metabolism, particularly those compounds used for fuel and anabolic processes (Hamad and Travis 2006). Chronic exposures to altitude however, are not feasible for many individuals, most of whom reside at sea level, for whom increases in metabolic rate and improvements in body mass and its distribution between fat and lean tissue would be useful to improve health risk factors. The application of normobaric and hypobaric chambers permits the wider use of acute hypoxia in a controlled setting, facilitating research into potential health benefits, including loss of body mass, especially fat.

Findings from experimental study 2 (Chapter 5) in this thesis observed an increased RMR and a greater proportional reliance on carbohydrate utilisation during an acute, severe exposure to $\text{FiO}_2:0.12$. Increases in lipolysis demonstrated by increased circulating FFA and TAG were also observed suggesting a metabolic perturbation during a short exposure. Previously a shift towards greater fat oxidation following exposure to an acute normobaric hypoxic has been observed (Workman and Bassett 2012) indicating that short, controlled, passive hypoxia may be sufficient to increase metabolic rate and increase reliance on lipids for fuel post-exposure. The study from Workman and Bassett hypothesised that a shift toward the utilisation of lipid sources would occur, following a greater dependency on glucose during exposure to hypoxia, (as found in study 2/ Chapter 5), in a response not dissimilar to the metabolic disruption observed following an exercise bout. The effects of acute exposure to hypoxia followed by moderate intensity exercise in normoxia however has not, to date been investigated. A prior hypoxic exposure causing an increase in EE and a greater reliance on fat oxidation following exposure to hypoxia may result in a greater reliance on fat sources during a subsequent exercise bout and thus a larger proportion of calories expended from fat sources.

Plasma fatty acids and TAG concentrations may increase following a high fat meal, termed a postprandial hyperlipidaemia, which in the long term may be associated with an increased risk of CVD (Zhang et al 1998). High blood lipids offer a diagnostic property for identifying metabolic syndrome symptoms, including insulin resistance, glucose intolerance and hypertension (Petitt et al., 2003) and

high levels of postprandial TAG are proposed as a potential CVD risk factor (Zhang et al 1998) . The effect of reduced FiO_2 on free fatty acid (FFA) appearance and uptake is complex and dependent upon, amongst other factors, energy intake and the energy balance of individuals (Barnholt et al., 2006; Roberts et al., 1996). Despite this, the effect of an acute bout of hypoxic exposure following a high lipid meal is not well researched. A reduction of postprandial hyperlipidaemia or improved lipid concentration control is likely to provide health benefits by decreasing metabolic syndrome symptoms and lowering cardiovascular disease risks.

Regular physical activity and exercise is important for successful management of body weight as it provides a means for increasing EE (Donnelly et al., 2004) thus helping to sustainably adjust energy balance. Increases of up to 8% in resting metabolic rate, in response to aerobic training, have been seen (Donnelly et al., 2004). Moderate intensity exercise (59-64% of $\dot{V}\text{O}_2$ max in trained and 47 – 52% in untrained populations) has been shown to induce the highest level of fat oxidation (Achten and Jeukendrup 2004). Previous reports have demonstrated the additional beneficial effect of exercise training in hypoxic conditions in reducing body mass and body fat percentage (Haufe et al., 2008; Netzer et al., 2008; Wiesner et al., 2010). Less is known, however, concerning the combination of rest in hypoxic conditions coupled with subsequent exercise in a normoxic environment on measures of lipid utilisation, EE and substrate partitioning. A preceding passive acute hypoxic exposure may increase EE, followed by a shift towards greater fat use (Workman and Basset 2012) during subsequent normoxic exercise as a consequence of the prior hypoxic exposure and increased fat content from a high lipid meal. A greater EE induced through hypoxic exposure and exercise combined with a post-hypoxic shift to greater lipid utilisation will likely reduce adiposity and enhance weight loss in a long term intervention. Aerobic exercise has also been shown to induce beneficial effects on postprandial lipaemia through increases in lipoprotein lipase expression and activity (Zhang et al., 1998) and the potential for greater oxidation of fat.

Meteorin-like is a recently identified and characterised hormone, made in skeletal muscle and present in the circulation. Its concentrations are affected by physiological stimuli, including physical activity and its release leads to the conversion of white adipose tissue to brown fat (Rao et al., 2014). Meteorin-like protein has been identified as a key PGC-1 α target gene and there is increased expression in human muscle and in mice following concurrent or eccentric exercise (Rao et al., 2014).

Meteorin-like expression is also increased following acute cold exposure (six hours at 4°C and 24 hours at 4°C) (Rao et al., 2014). Meteorin-like protein aids cold-induced thermogenic responses, suggesting a role in the metabolic adaptations to cold temperatures. Furthermore increases in Metrnl, through the delivery of Metrnl-expressing adenoviral vectors, increase whole body EE associated with the browning of the white fat depots and an improvement in glucose tolerance in obese and diabetic mice, five days after injection (Rao et al., 2014). An improvement in glucose tolerance coupled with

increases in EE has previously been observed in humans with metabolic syndrome following a three week stay at 1,700m (Schobersberger et al., 2003).

Similarities between the physiological response upon exposure to hypoxia and cold exist highlighting the potential effect of hypoxia/altitude on levels of Meteorin-like. An increase in sympathetic nervous system activation exists upon exposure to conditions of both cold and hypoxia. This increase in sympathetic drive is a suggested mechanism for observed increases in metabolic rate upon exposure to high altitude (Butterfield et al. 1992; Mawson et al., 2000). An improvement in glucose tolerance in obese individuals exposed to altitude has previously been observed (Schobersberger et al., 2003). Similarly, in mice delivered with Meteorin-like expressing adenoviral vectors improved glucose tolerance was reported (Rao et al., 2014). Such findings support the rationale for the examination of Meteorin-like during exposure to hypoxia. An increased level of Metrnl could have implications for “browning” of fat stores in the body and may serve as a suggestion for the mechanisms surrounding loss of fat previously observed at altitude. Increased Metrnl may also have implications for increasing resting metabolic rate and consequently weight loss in clinical populations. There is currently no information on the acute effects or responses of hypoxia on Metrnl.

The primary aims of the study were to 1) quantify changes in EE and fuel use in response to acute hypoxia. This will be addressed through comparison of HYPCON and NORMCON trials. 2) Investigate the effects of a moderate intensity exercise bout following hypoxia and a HLD on EE and fuel use. This will be addressed through comparison of HYP and NORM and between HYP and HYPCON trials. Finally 3) to examine the response to hypoxia, moderate intensity exercise and a HLD on plasma concentrations of Metrnl. This will be addressed through comparison of all trials. It was hypothesised that an acute bout of hypoxia would increase EE during rest. Furthermore it was hypothesised an increase in fat through a HLD in combination with hypoxia would increase fat use during subsequent exercise. Finally it was hypothesised that the combination of acute hypoxia and moderate intensity exercise may act together to promote the release of Metrnl and increase fat use as a fuel. This study fits within the thesis by incorporating the acute exposure in study 2 (Chapter 5) with moderate intensity exercise. This comes prior to the investigation of metabolic markers during and following a more prolonged period of altitude stay in study 4 (Chapter 7).

6.3 MATERIALS AND METHODS

PARTICIPANTS

Eight males [22 ± 5 years of age (mean \pm SD), body mass 75.7 ± 9.1 kg, height 178 ± 7 cm and BMI 24 ± 4 (kg/m^2)] agreed to partake in the study and complied with all criteria for participation [(3.5.1) Medical criteria and recruitment and (3.6) Pre-trial diet and exercise standardisation and (3.8) Hydration assessment]. Prior to the undertaking of the experimental trials volunteers attended the laboratories whereby their anthropometric data was collected [(3.11) Anthropometric assessment].

Participants were instructed to complete a 12 hour overnight fast prior to testing, having consumed their last meal before 20:00 on the preceding evening and to maintain their normal diet throughout the time period of testing.

EXPERIMENTAL DESIGN

The study required participants to attend the laboratory on five occasions. Three trials required participants to consume a HLD at min 0 (CON, HYP, NORM). No HLD was consumed during the remaining two trials (HYPCON and NORMCON). Prior to each trial two baseline values were taken (PRE) (Figure 6.1). Participants were exposed to hypoxia conditions in a purpose built nitrogen enriched hypoxic chamber (Altitude Centre, London). The chamber environment was controlled for each trial [(3.12.2) Controlling the hypoxic environment]. Trials were randomised for order. The experimental design is illustrated in Figure 6.1. A description of each experimental design is outlined below.

CON. Participants were required to ingest a high lipid meal prior to a resting period of 240 minutes.

HYP. Participants were required to ingest a high lipid meal prior to a resting period of 60 minutes at $FiO_2:0.12$ before completing 60 minutes of cycling exercise at 60% HRR in $FiO_2:0.21$. A final 120 minute period of rest in $FiO_2:0.21$ was then undertaken.

NORM. Participants were required to ingest a high lipid meal prior to a resting period of 60 minutes at $FiO_2:0.21$ before completing 60 minutes of cycling exercise at 60% HRR in $FiO_2:0.21$. A final 120 minute period of rest in $FiO_2:0.21$ was then undertaken.

HYPCON. Participants were required to rest for a period of 60 minutes at $FiO_2:0.12$ before completing 60 minutes of cycling exercise at 60% HRR in $FiO_2:0.21$ in a fasted state. A final 120 minute period of rest in $FiO_2:0.21$ was then undertaken.

NORMCON. Participants were required to rest for a period of 60 minutes at $FiO_2:0.21$ before completing 60 minutes of cycling exercise at 60% HRR in $FiO_2:0.21$ in a fasted state. A final 120 minute period of rest in $FiO_2:0.21$ was then undertaken.

Equation 6.1: Calculation of Age Predicted Maximum Heart Rate (HR_{max}) (Tanaka et al. 2001)

$$HR_{max} = 208 - 0.7 \times \text{age (years)}$$

Equation 6.2: Calculation of Heart Rate Reserve (HRR) (Karvonen et al. 1957)

$$HRR = HR_{max} - HR_{rest}$$

Where HR_{max} is age predicted maximum heart rate and HR_{rest} is resting heart rate

$$60\% \text{ HRR} = \text{HRR} \times 0.60 + \text{HR}_{\text{rest}}$$

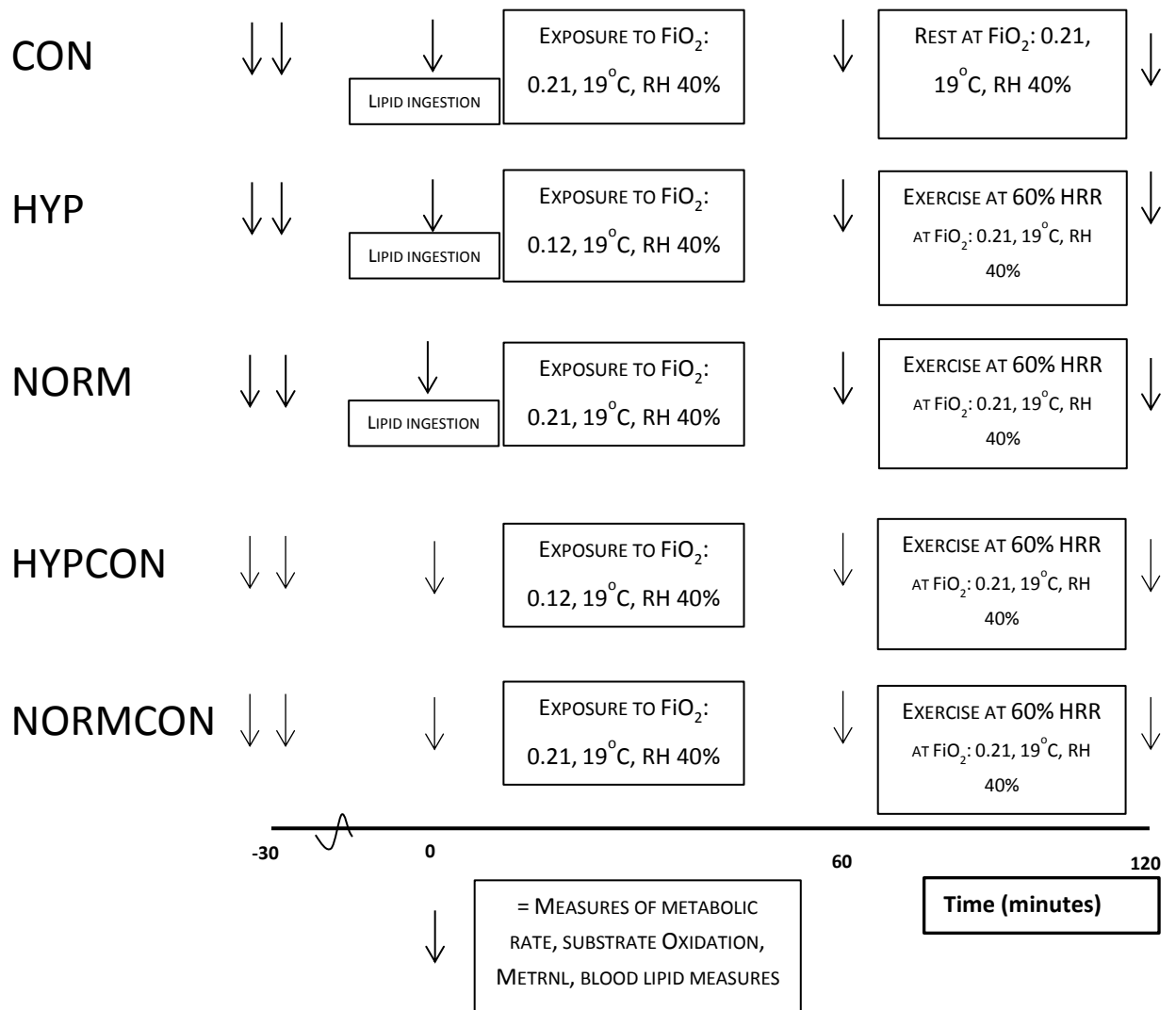


FIGURE 6.1: Schematic of experimental design.

Notes: Control (CON), Hypoxia and exercise with lipid ingestion (HYP), Normoxia and exercise with lipid ingestion (NORM), Hypoxia and exercise with no lipid ingestion (HYPCON), Normoxia and exercise with no lipid ingestion (NORMCON), heart rate reserve (HRR), fraction of inspired Oxygen (FiO_2), relative humidity (RH).

GAS MEASURES AND PHYSIOLOGICAL DATA

Expired gas was collected and analysed using the Douglas bag method [(3.13.1) Douglas bag metabolic gas analysis]. Resting metabolic rate and substrate utilisation were determined indirectly via non-protein respiratory exchange ratio (RER) which was determined through expired gas. Non-protein RER assumes constant protein oxidation. Oxidation rates of fat and carbohydrate (CHO) were

calculated according to the following equations outlined (Peronnet and Massicotte 1991) [(3.13.3) Calculations derived from cardiopulmonary measures].

Throughout exposure to hypoxia HR and SpO_2 were monitored and every 30 min were recorded using a HR monitor (Polar, Finland) and a fingertip pulse oximeter (Nonin 2500, Nonin Medical Inc., USA) respectively. Peripheral arterial oxygen saturation was measured at each 30 min interval in normoxia. [(3.13.5) Heart rate and (3.13.4) Peripheral artery oxygen saturation]. TEE was calculated by assuming constant expenditure at each time point for 30 min.

BLOOD MEASURES OF LIPID METABOLISM

Two baseline venous blood samples were taken [(3.17.1) Cannulation] on arrival to the laboratories (PRE1 and PRE2) separated by 30 minutes. Subsequently samples were taken at min 0 and from then on at each 60 min time point. With plasma separated [(3.17.2) Blood plasma separation] FFA, TAG and MetrnI were analysed [(3.17.3) Analysis of FFA and (3.17.4) Analysis of TAG and (3.17.5) Analysis of Meteorin-like].

MONITORING FOR SYMPTOMS OF ACUTE MOUNTAIN SICKNESS (AMS)

During exposure to FiO_2 : 0.12 a modified Lake Louise Questionnaire (LLQ) (Roach et al., 1993) was used to monitor symptoms of AMS at every 10 minute interval [3.14.1) Lake Louis Questionnaire score].

TEST MEAL

Participants were provided with a high-lipid meal in a liquid form and instructed to consume the drink in less than 10 minutes. The meal consisted of a combination of 180 mL double cream and 110 g of ice cream. Similar meals have been successfully used in previous studies to induce postprandial hypertriglyceridemia (PHTG) (Zhang et al., 2007). The HLD provided 883 kcal, 73 g fat, 45 g CHO and 9 g protein.

STATISTICAL ANALYSIS

All data were checked for normality and sphericity [(3.15.2.1) Normal distribution and (3.15.2.2) Sphericity] and were adjusted using the Huynh-Feldt method. All data were analysed using a standard statistical package (SPSS version 20 for Windows 7).

Two-way analysis of variance (ANOVA) with repeated measures was used to identify main effect of the condition and or the time point for blood measures (3 x 6) and for other variables (3 x 9). Main effects were followed up using Bonferoni pairwise comparisons comparing separate conditions, between time points and between conditions at individual time points. Effect sizes for main effects

and interactions are presented as partial eta squared (η^2), while differences between two related samples were evaluated through Cohen's d_{av} in accordance with Lakens (2013).

The area under the curve (AUC) values was calculated using the conventional trapezoid method. A general linear model, one-way, repeated measures analysis of variance was used to identify main effect of condition. Main effects were followed up using Bonferoni pairwise comparisons comparing separate conditions.

Equation 6.3: Calculation of Area under the Curve by the trapezoid method.

$$\text{AUC} = (Y_1 + Y_2 / 2) \times W$$

Where Y_1 is the height of one side of the trapezoid, Y_2 is the height of the other side of the trapezoid and W is the width of the trapezoid.

6.4 RESULTS

PHYSIOLOGICAL MEASURES

$\dot{V}O_2$ was different between conditions ($F_{(2, 12)} = 27.983, p \leq 0.001, \eta^2 = 0.823$). Hypoxic rest (HYP) increased $\dot{V}O_2$ by 1.26 ± 0.79 (+33% $p = 0.006, d = 1.50$) at 30 min and 0.89 ± 0.49 ml.kg.min⁻¹ (+24% $p = 0.004, d = 0.98$) at 60 min compared to NORM during which participants rested in $FiO_2:0.21$. Cycling exercise for 60 min induced increases in $\dot{V}O_2$ in HYP ($p = 0.03, d = 3.13$) and NORM ($p \leq 0.001, d = 4.99$) during which participants completed 60 minutes of moderate intensity cycling compared to CON. No difference in $\dot{V}O_2$ between HYP and NORM was observed ($p = 0.157$). Exercise induced increases of 14.91 ± 8.27 ml.kg.min⁻¹ and 22.44 ± 8.17 ml.kg.min⁻¹ at 120 min in HYP and NORM respectively compared to CON.

$\dot{V}CO_2$ was different between conditions ($F_{(2, 12)} = 27.336, p \leq 0.001, \eta^2 = 0.820$). Hypoxic rest increased $\dot{V}CO_2$ by 1.27 ± 0.64 ml.kg.min⁻¹ at 30 min ($p = 0.027, d = 1.33$) and 1.15 ± 0.38 ml.kg.min⁻¹ at 60 min ($p = 0.008, d = 1.45$) respectively compared to rest in $FiO_2: 0.21$ in NORM. Increases in $\dot{V}CO_2$ were also induced by cycling at 120 min in both HYP ($p = 0.002, d = 3.48$) and NORM ($p < 0.001, d = 4.87$) compared to a period of rest in CON.

Heart rate was different between conditions ($F_{(16, 96)} = 64.576, p < 0.001, \eta^2 = 0.915$). Increases of 18 ± 9 b.min⁻¹ (+21% $p = 0.002, d = 1.44$) and 14 ± 5 b.min⁻¹ (+18% $p < 0.001, d = 1.22$) were reported at 30 and 60 min respectively following hypoxic rest compared to rest in $FiO_2:0.21$ in NORM. Increases in HR during cycling at 90 and 120 min in HYP and NORM were observed compared to CON in which no exercise was undertaken ($p < 0.05$). Heart rate remained elevated in HYP post-exercise at minute 150 ($p = 0.04, d = 0.93$) and in NORM at 150 min ($p = 0.017, d = 0.97$), 180 min ($p = 0.03, d = 1.08$) and 240 min ($p = 0.034, d = 0.79$) compared to CON. Greater HR was observed in NORM following a HLD in

comparison to fasted measures in NORMCON ($p = 0.002$, $np^2 = 0.807$) at 30 (+ 9%, $p = 0.039$, $d = 0.64$) and 60 min (+ 11%, $p = 0.006$, $d = 0.73$).

\dot{V}_E was different between conditions ($F_{(3, 18)} = 5.129$, $p = 0.010$, $np^2 = 0.461$). Upon exposure to hypoxia increases of \dot{V}_E to 3.4 ± 2.9 L.min⁻¹ were observed compared to rest at FiO₂:0.21 in NORM at min 30 (+38%, $p = 0.02$). Increases in \dot{V}_E during cycling exercise at minutes 90 and 120 in HYP and NORM were observed compared to CON in which no exercise was undertaken ($p < 0.05$). Increases of 29.9 ± 10.6 L.min⁻¹ and $29. \pm 10.6$ L.min⁻¹ were observed respectively.

Peripheral arterial oxygen saturation was different between conditions ($F_{(2, 12)} = 104.147$, $p < 0.001$, $np^2 = 0.946$). Decreases at min 30 and 60 in hypoxia ($p < 0.001$, $d = 8.5$ and $p < 0.001$, $d = 6.8$) were observed so that individuals were exhibiting values of $81 \pm 4\%$ following 60 min of rest in hypoxia compared to $98 \pm 1\%$ following normoxic rest. Peripheral arterial oxygen saturation was lower at 90 (-2%) ($p = 0.001$, $d = 7.2$), 120 (-2%) ($p = 0.005$, $d = 5$), 150 (-1%) ($p = 0.03$, $d = 1$) and 180 (-1%) ($p = 0.012$, $d = 2$) min, in HYP compared to CON. No differences in pulse oximetry were observed between HYP and NORM conditions from 90 to 240 min ($p > 0.05$). No difference between HYP and HYPCON or between NORM and NORMCON were observed for SpO₂ throughout the session ($p > 0.05$).

ENERGY EXPENDITURE AND SUBSTRATE OXIDATION

Addressing the aim in which the effects of acute hypoxia on fuel use and resting metabolic rate (RMR) were examined; Calculated EE was different between conditions ($F_{(2, 12)} = 27.790$, $p \leq 0.001$, $np^2 = 0.822$), HYP induced greater EE at rest compared to NORM at 30 (+ 35%, $p = 0.006$, $d = 2.0$) and 60 min (+ 26% $p = 0.002$, $d = 1.44$) so that individuals were expending 30 ± 19 and 22 ± 11 kcal.hr⁻¹ more at these time points respectively. EE at rest in HYP was greater than that in CON at 30 (+ 26%, $p = 0.019$, $d = 1.45$) and 60 min (+ 35%, $p = 0.009$, $d = 1.82$) so that individuals were expending 22 ± 19 kcal.hr⁻¹ and 30 ± 21 kcal.hr⁻¹ more at these time points respectively.

Respiratory exchange ratio was not different between conditions ($F_{(2, 12)} = 2.882$, $p = 0.095$, $np^2 = 0.324$) although a move toward an increase in RER values during hypoxic exposure (NORM 0.76 ± 0.1 vs HYP 0.89 ± 0.16 , $d = 0.7$) was observed. FAT oxidation differed between conditions ($F_{(2, 12)} = 28.618$, $p < 0.001$, $np^2 = 0.827$). Following 60 min rest HYP induced greater FAT oxidation values of 0.11 ± 0.08 g.min⁻¹ compared to 0.06 ± 0.03 g.min⁻¹ in NORM ($p = 0.002$, $d = 0.91$) and 0.07 ± 0.05 g.min⁻¹ in CON ($p = 0.004$, $d = 0.62$). HYP rest induced increases of 12% compared to PRE values ($p = 0.003$, $d = 1.11$) whereas rest in normoxic conditions resulted in -6% change ($p = 0.675$, $d = 0.29$) compared to PRE values.

Carbohydrate oxidation differed between conditions ($F_{(2, 12)} = 28.618$, $p = 0.003$, $np^2 = 0.620$). Increases in CHO oxidation were observed at 60 min (0.62 ± 0.10 g.min⁻¹) compared to PRE (0.41 ± 0.18 g.min⁻¹) in HYP ($p = 0.046$, + 37%, $d = 1.91$). Carbohydrate utilisation following 60 minutes of normoxic rest remained stable (Figure 6.2).

Addressing the aim in which the effects of a high fat meal (HLD) (883 kcal, 73g fat) on fuel use and resting metabolic rate (RMR) were examined; Ingestion of a HLD had no effect on EE in HYP compared to HYPCON or in NORM compared to NORMCON ($p > 0.05$). Ingestion of a HLD alone did not alter CHO oxidation in HYP compared to HYPCON or in NORM compared to NORMCON ($p > 0.05$). Ingestion of a HLD alone did not alter FAT oxidation in HYP compared to HYPCON or in NORM compared to NORMCON ($p > 0.05$). Ingestion of a HLD had no effect on TOTAL EE in any condition.

Addressing the aim in which the effects of subsequent exercise on EE and fuel use were examined; during subsequent exercise a greater EE was observed in HYP and NORM compared to CON. At min 90 and 120 increases of $7.40 \pm 3.25 \text{ kcal}\cdot\text{min}^{-1}$ and $6.60 \pm 2.15 \text{ kcal}\cdot\text{min}^{-1}$ in HYP ($p < 0.05$) and $6.21 \pm 3.24 \text{ kcal}\cdot\text{min}^{-1}$ and $8.26 \pm 2.87 \text{ kcal}\cdot\text{min}^{-1}$ in NORM ($p < 0.05$) respectively were observed with individual responses ranging from a 77-91% increase in HYP and from a 80-95% increase in NORM at 120 mins compared to CON. No differences in EE were recorded between exercise conditions during cycling ($p > 0.05$). During recovery time points no differences in EE were observed between conditions ($p > 0.05$) (Figure 6.2).

Respiratory exchange ratio was not different between conditions ($F_{(2, 12)} = 2.882$, $p = 0.095$, $np^2 = 0.324$) although a move toward an increase in RER values between rest and exercise in normoxia (REST 0.73 ± 0.12 . vs. EXERCISE 0.84 ± 0.06 , $d = 1.16$) was observed.

During subsequent exercise individuals were utilising $0.2 \pm 0.2 \text{ g}\cdot\text{min}^{-1}$ and $0.39 \pm 0.21 \text{ g}\cdot\text{min}^{-1}$ more FAT in HYP and NORM compared to CON ($p < 0.05$). Exposure to hypoxia for 60 minutes prior to cycling exercise did not induce alterations in FAT utilisation in HYP compared to NORM ($p > 0.05$) (Figure 6.2).

Individuals were utilising $0.68 \pm 0.66 \text{ g}\cdot\text{min}^{-1}$ more CHO in HYP and $0.81 \pm 0.95 \text{ g}\cdot\text{min}^{-1}$ more CHO in NORM compared to CON at minute 120 ($p < 0.05$). A 60 minute hypoxic exposure prior to cycling exercise did not induce alterations in CHO utilisation in HYP compared to NORM ($p > 0.05$) (Figure 6.2).

TOTAL EE was different between conditions ($F_{(2, 12)} = 29.489$, $p \leq 0.001$, $np^2 = 0.831$). Individuals were expending $652 \pm 113 \text{ kcal}$ and $714 \pm 179 \text{ kcal}$ more in HYP and NORM respectively over the 4 hour study period compared to CON (Figure 6.3).

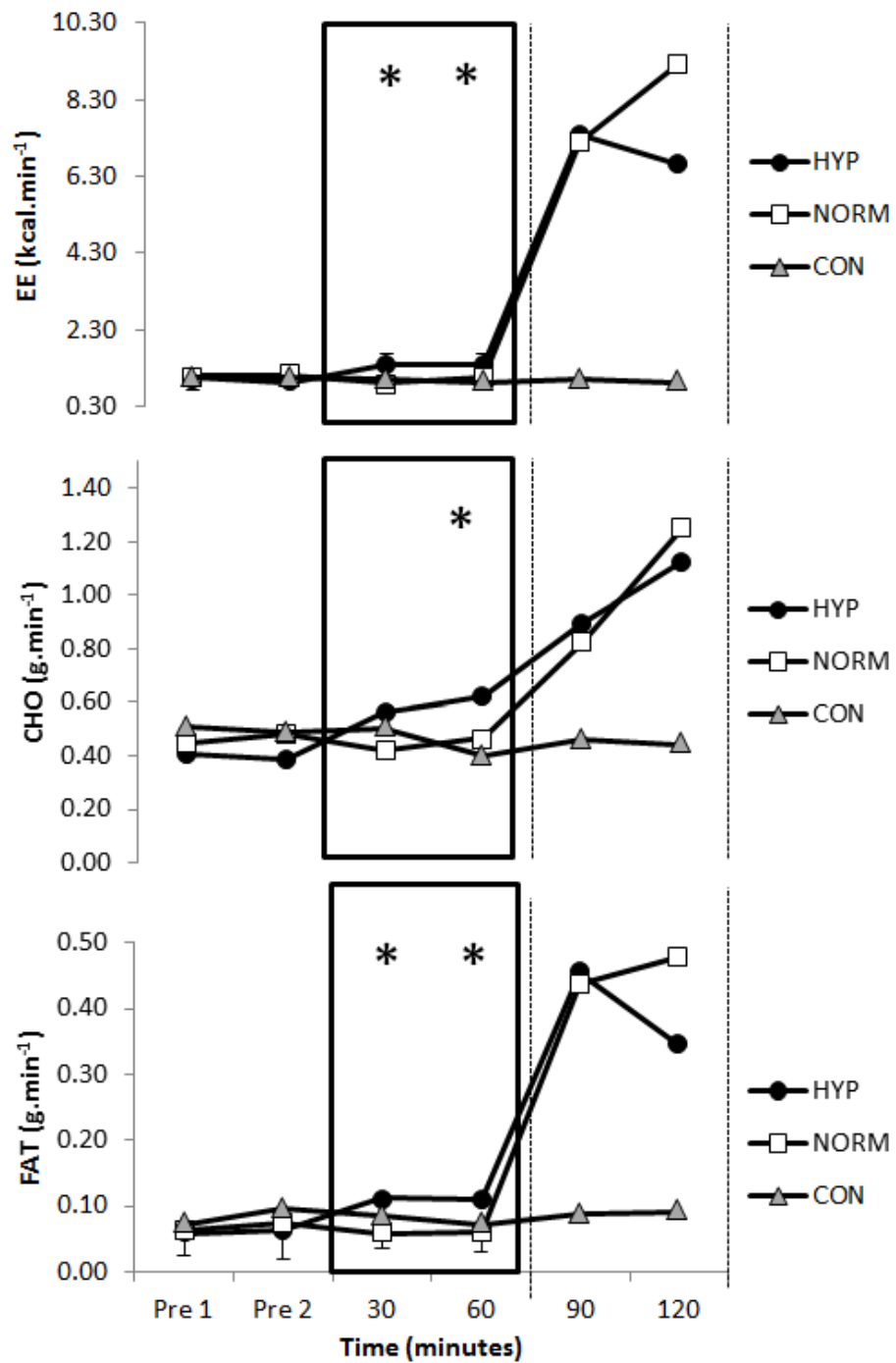


FIGURE 6.2: Energy expenditure (EE) and carbohydrate (CHO) and fat (FAT) oxidation in hypoxia and exercise (HYP), normoxia and exercise (NORM) and control (CON) conditions (mean \pm SD).

Notes: * denotes differences between HYP and NORM conditions. All significance symbols correspond to $p < 0.05$. Boxed outline indicates exposure to $\text{FiO}_2:0.12$ in HYP condition. Dashed line indicates cycling exercise in HYP and NORM. Error bars removed for clarity.

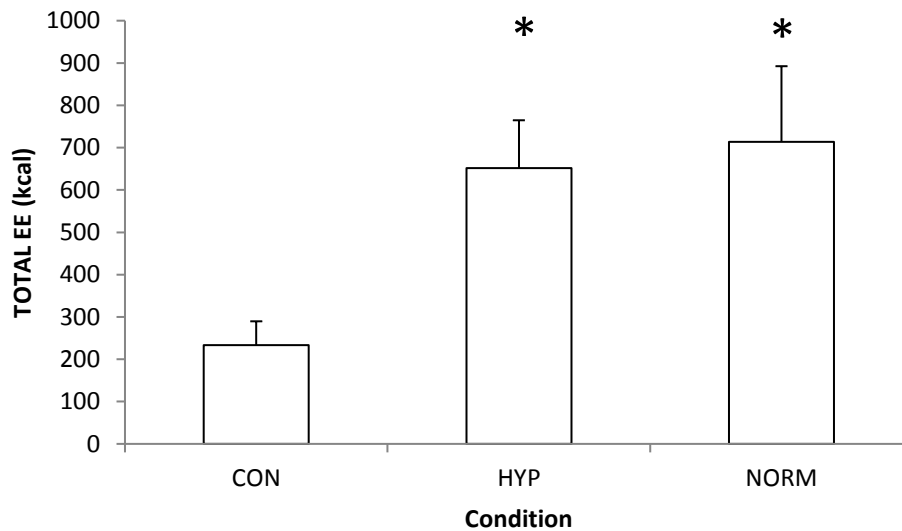


FIGURE 6.3: Comparison of total EE between hypoxia and exercise, normoxia and exercise and control conditions. Notes: Total energy expenditure (TEE), hypoxia and exercise (HYP) normoxia and exercise (NORM), control (CON). * indicates differences compared to CON condition. All significance symbols correspond to $p < 0.05$.

BLOOD MEASURES RESPONSES

Addressing the aim to measure the effects of hypoxia, a HLD and exercise on Meteorin-like concentration; Metrnl was unaltered by condition ($F_{(2, 10)} = 3.469$, $p = 0.82$, $np^2 = 0.464$), and throughout the testing period ($F_{(5, 25)} = 1.216$, $p = 0.338$, $np^2 = 0.233$) (Figure 6.4).

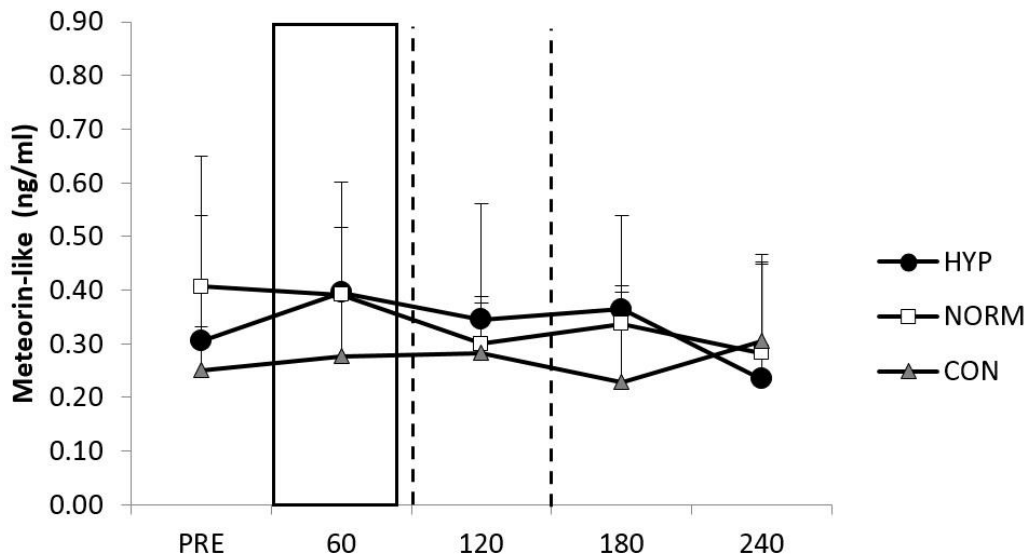


FIGURE 6.4: Meteorin-like in control, hypoxia and exercise, and normoxia and exercise conditions (mean \pm SD).

Notes: Boxed outline indicates exposure to $\text{FiO}_2:0.12$ in HYP. Dashed line indicates cycling exercise in HYP and NORM. Meteorin-like (MetrnI) control (CON) hypoxia and exercise (HYP) normoxia and exercise (NORM)

To address the aim of acute hypoxia and a high fat meal (HLD) (883 kcal, 73g fat) on metabolic blood measures; ingestion of a HLD induced increases in FFA ($p = 0.001$, $\eta^2 = 0.882$) and TAG concentration ($p = 0.001$, $\eta^2 = 0.540$) in all conditions compared to fasting measures. Peak increases in AUC values of $0.28 \text{ mmol.L}^{-1}\cdot\text{hr}$ for FFA and $0.15 \text{ mg}\cdot\text{hr/dL}^{-1}$ for TAG were observed following the meal in CON.

Free fatty acid AUC values were greater following exposure to hypoxia compared to normoxia. This was observed both when individuals had consumed a HLD ($p = 0.02$) and when fasted ($p = 0.04$). AUC values in HYP, in which participants rested in $\text{FiO}_2:0.12$ prior to moderate intensity exercise, were $0.56 \pm 0.16 \text{ mmol.L}^{-1}\cdot\text{hr}$ compared to $0.42 \pm 0.10 \text{ mmol.L}^{-1}\cdot\text{hr}$ in NORM, during which participants rested in $\text{FiO}_2:0.21$ prior to moderate intensity exercise, which equated to a $25 \pm 3\%$ difference. Fasted AUC values in HYPCON were $0.39 \pm 0.04 \text{ mmol.L}^{-1}\cdot\text{hr}$ compared to $0.25 \pm 0.03 \text{ mmol.L}^{-1}\cdot\text{hr}$ in NORMCON.

No difference in FFA values were observed between exposure to hypoxia following a HLD and exposure to hypoxia when fasted ($p = 0.061$). Similarly no difference between NORM (ingestion of a HLD) and NORMCON (fasted) trials were observed ($p = 0.152$). Measures of TAG and FFA for conditions of HYP, NORM and CON are presented in Figure 6.5.

Addressing the aim in which the effects of subsequent exercise on metabolic blood measures were examined; moderate intensity exercise reduced TAG AUC values in HYP and NORM compared to a period of rest rather than exercise in CON ($p = 0.035$). Triglyceride AUC values were greater in CON ($0.41 \pm 0.05 \text{ mg.hr/dL}^{-1}$) compared to HYP ($0.30 \pm 0.06 \text{ mg.hr/dL}^{-1}$) and NORM ($0.29 \pm 0.05 \text{ mg.hr/dL}^{-1}$) which equated to a $37 \pm 17\%$ and a $27 \pm 12\%$ difference respectively.

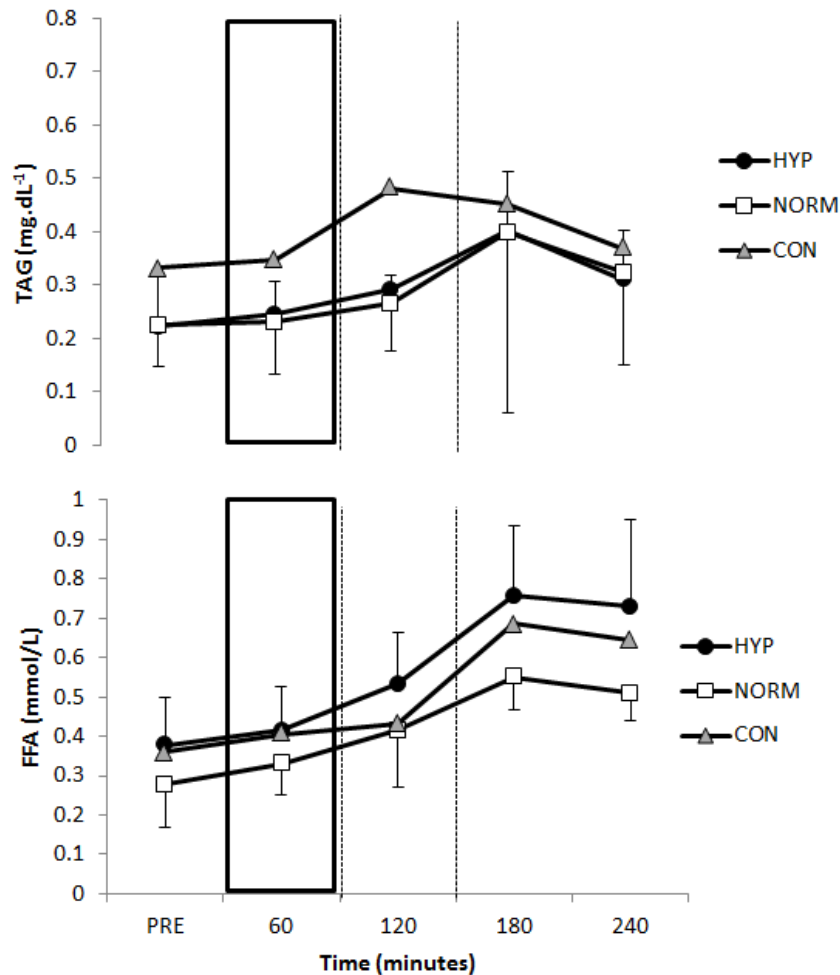


FIGURE 6.5: Triglycerides and free fatty acids in control, hypoxia and exercise and normoxia and exercise conditions

Notes: Data displayed as mean \pm SD. Boxed outline indicates exposure to $\text{FiO}_2:0.12$ in HYP. Dashed line indicates cycling exercise in HYP and NORM. A high lipid meal was ingested at minute 0. Triglycerides (TAG), free fatty acids (FFA), control (CON), hypoxia and exercise (HYP), normoxia and exercise (NORM).

6.5 DISCUSSION

The present study investigated the acute effects of hypoxia coupled with moderate intensity exercise with and without a high fat meal on metabolic effects including EE, substrate use and MetrnI concentration. These data support the hypotheses that an acute, passive hypoxic exposure is sufficient to increase EE through increases in FAT and CHO use, with predominance from CHO sources, in healthy humans. These data also show that exposure to hypoxia alone raises FFA concentration as does a high fat meal. A prior hypoxic exposure did not induce acute alterations in substrate oxidation during a subsequent exercise bout, suggesting that greater fat oxidation does not occur during moderate intensity exercise as it has been shown to do during post-hypoxic rest (Workman and Basset 2012). Moderate intensity exercise was seen to be useful for lowering postprandial hypertriglyceridaemia following a high fat meal, demonstrated through lower AUC TAG values in both exercise groups in comparison to a control after hypoxic exposure and during normoxia. This is the first study to show no acute effect of exercise, hypoxia or fat ingestion on MetrnI.

ENERGY EXPENDITURE AND SUBSTRATE OXIDATION

Acute hypoxic exposure in the present study was seen to induce increases in EE, as per previous investigations (Workman and Basset 2012) and study 2 (Chapter 5) of this thesis, highlighting the effect of environmental conditions on metabolism and substrate use. An increase in the present study of $0.4 \pm 0.2 \text{ kcal}\cdot\text{min}^{-1}$ following acute exposure compared to approximately $0.3 \text{ kcal}\cdot\text{min}^{-1}$ in work from Workman and Basset (2012) highlights similar absolute increases across the two protocols in which similar SpO_2 values were induced by the hypoxic stimulus ($81 \pm 4\%$ and $81 \pm 2\%$) and similar to the results from study 2 (Chapter 5) of this thesis. Greater percentage increases in EE values were reported in the present study of 26% compared to Workman and Bassett (2012) which may be explained by lower initial EE values in the current investigation. Absolute increases of $22 \pm 11 \text{ kcal}\cdot\text{hr}^{-1}$ from a single bout of hypoxic exposure translates to approximately $521 \pm 273 \text{ kcal}\cdot\text{day}^{-1}$ which is similar to the $500 \text{ kcal}\cdot\text{day}^{-1}$ previously calculated as the extra required energy intake to maintain body mass in males at 4,300m (Brooks, 2014; Marriott and Carlson, 1996). Potential mechanisms for increased metabolic rate following hypoxic exposure include an increase in sympathetic drive (Mawson et al., 2000), indicated by an increased HR during hypoxia in the present study (+ 18%), and induced higher basal noradrenaline levels (Urdampilleta et al., 2012), not measured in the present study, which may explain current findings. The increased EE was reported in conjunction with a greater reliance on both CHO and FAT use throughout hypoxic exposure. Although the effect of environmental hypoxia on glycolytic capacity is debated, an up-regulation of the transcription factor hypoxic inducible factor in cultured cells leads to increased glycolysis (Semenza et al., 1994) as an adaptive response to altitude when oxygen availability is limited. An increase in glycolysis would improve oxygen economy (Horscroft and Murray 2014) as metabolism of glucose via glycolysis requires less oxygen compared to oxidative phosphorylation. This metabolic shift however, has also been described as energy inefficient as only two ATP are formed for each glucose molecule and six ATP are consumed for every two molecules of

lactate converted to glucose. This “inefficiency” may itself be linked to an increased metabolic rate (Palmer and Clegg 2014).

An increase in both CHO and FAT oxidation at rest highlights a general metabolic perturbation following hypoxia inducing increased EE. Although both FAT and CHO use was seen to increase, in relation to calories consumed a greater proportional increase in CHO was observed following a 60 minute rest in hypoxia compared to FAT. Increases in the utilisation of carbohydrate derived fuel sources of glucose, glycogen and lactate are seen consistently at altitude in men and women upon acute exposure (Brooks et al., 1991). Previous work has also demonstrated greater reliance on lipid metabolism and a dampening of the altitude induced increase in glucose availability when individuals are energy deficient and exposed to hypoxia in an attempt to spare the limited supply of carbohydrates (Roberts et al., 1996). A 37% increase in CHO oxidation was observed at 60 minutes following exposure to $\text{FiO}_2:0.12$ in combination with a 12% increase in FAT oxidation. Furthermore an observed trend for an increase in RER values during hypoxic exposure (NORM 0.76 ± 0.1 . vs HYP 0.89 ± 0.16 , $d = 0.7$) highlights the likelihood of an increased contribution of CHO derived fuels resulting in an increased metabolic rate (Horscroft and Murray 2014). This trend, although supported by previous work, did not reach statistical significance and must therefore be interpreted with caution in light of an observed increase in \dot{V}_E .

Previous work from Workman and Basset, (2012) reported an immediate shift towards greater lipid oxidation following both acute and short-term hypoxic exposures. This shift occurs following a greater dependency on glucose during hypoxia and is based on the metabolic perturbation induced by reduced FiO_2 . A repayment of the oxygen deficit similar to an elevation in metabolism post-exercise is, in part, responsible for this shift. Another possible mechanism for this post-exposure shift toward lipid oxidation is a greater autonomic neuroendocrine stimulation of lipolysis reflected by an increase in a marker of oxidative stress, malondialdehydes, which has been reported during hypoxic exercise compared to normoxic exercise (Pialoux et al., 2006). Post-exercise metabolism is fuelled by higher lipid usage which is partially stimulated by increased catecholamine release. Workman and Bassett, (2012) suggest that post-exposure responses including a shift to lipid sources following a greater shift to glucose dependence during hypoxic exposure is a metabolic pattern that is similar, but slightly different, to post-exercise oxygen consumption deficit and its related mechanisms. Furthermore raised lipid oxidation through the transcription co-activator peroxisome proliferator-activated receptor- γ co-activator-1 α following exercise training in hypoxia has been observed (Wiesner et al., 2010). This process allows PGC1 α , a central inducer of mitochondrial biogenesis (Haufe et al, 2008), to play a key role in mediating adaptive regulation of muscle fatty acid oxidation (Wiesner et al., 2010). In support of these findings the current study found an increase in lipid oxidation following hypoxic exposure; however this was seen only in line with increased EE and in conjunction with an increase in CHO utilisation also. Interestingly however a greater increase in FAT oxidation (4 fold increase) proportional to calories was observed compared to the observed increase in CHO (2 fold increase)

oxidation during moderate intensity exercise across conditions. Nevertheless our study was unable to confirm our hypothesis that a prior exposure to hypoxia significantly increases FAT use during subsequent moderate intensity exercise in comparison to resting in normoxia. Discrepancies between findings may be due to the shorter hypoxic exposure administered in the present study, the fact that post-exposure exercise was undertaken immediately and a reflection of the moderate intensity of the cycling exercises.

TOTAL ENERGY EXPENDITURE AND BLOOD MEASURE RESPONSES

The lack of difference in TEE reported between exercise trials was most likely due to the cycling exercise being the same across trials and no expected change in efficiency thus inducing similar EE values despite differing metabolic rates prior to exercise. Furthermore any basal increase of EE induced by hypoxia is small in comparison with the changes elicited by exercise and unlikely to be measurable. However the cumulative effect over long periods of time of increased resting EE is likely to have a significant effect on fuel utilisation and body mass regulation. An increased hypoxic induced metabolic rate prior to exercise may therefore have little effect on substrate partitioning and total expended energy during exercise. Physical exercise has previously been shown to reduce postprandial lipaemia (Petitt and Cureton 2003; Katsanos et al. 2004; Plaisance et al. 2008) through an increase in the activity of lipoprotein lipase within the capillaries of the exercising muscles and a subsequent accelerated clearance and metabolism of circulating TAG (Kolifa et al., 2004). It has been suggested that a shortening of postprandial lipaemia may have beneficial health effects and may prevent the atherogenic process (Zhang et al., 2007). The impact of hypoxic exposure on lipolysis is a complex issue with some previous investigations reporting stimulation of lipolysis by hypoxia (Roberts et al, 1996). Increased plasma and liver TAG levels have been correlated to increased severity of FiO_2 , largely driven by increased hepatic secretion and decreased lipoprotein clearance (Jun et al., 2012). The decrease in lipoprotein clearance was highlighted by the delay in postprandial TAG clearance in hypoxic conditions, found in conjunction with a decreased uptake and oxidation of FFA. In contrast, the combination of exercise and hypoxia induced reductions in fasting TAG concentrations in lean healthy men whereas training alone had no such effect (Netzer et al., 2008).

A high fat meal was seen to increase FFA in the current study as well as increases due to hypoxia alone. This occurred when individuals were fasted and following a high fat meal. Moderate intensity exercise, used in the present study was effective at lowering postprandial hypertriglyceridaemia more than in control, non-exercised conditions, following a HLD. This was clear from lower TAG values, measured as the area under the curve. There were no significant effects of hypoxic exposure on this process, exercise with or without prior hypoxic exposure was equally effective in reducing lipid load in the blood. Furthermore TAG values reached a higher concentration in CON more quickly than both exercise groups with values at 120 minutes 54% and 40% higher in CON compared to HYP and NORM respectively.

This study adds to previous work concerning the regulation of Metrnl. Following hypoxic exposure and the combination of passive hypoxic rest and moderate intensity exercise in normoxic conditions there were no acute effects of exercise, hypoxia or fat ingestion on Metrnl. The rationale behind the current study was based on the facts that exposure to altitude and exercise have significant effects on body composition, fat mass and whole body metabolic processes that might be accounted for by changes in whole brown adipose tissue. Meteorin-like protein is present in the circulation and induced, in skeletal muscle, by exercise and in adipose tissue following cold exposure. We hypothesised that exposure to hypoxia may also induce changes in Metrnl and play a role in the mechanisms leading to loss of fat mass at altitude.

Brown adipose tissue is associated with the maintenance of core temperature with studies demonstrating that exposure to cold conditions expands thermogenic capacity by increasing the amount of brown adipocytes in brown adipose tissue (Hao et al., 2014) and by recruiting “beige cells in white adipose tissue (Cannon, 2004). This is considered useful as white adipose tissue is believed to contribute to chronic inflammation in obesity and disorders such as insulin resistance and type 2 diabetes through the secretion of adipokines (Lago et al., 2009). Meteorin-like protein, rather than having a direct action on adipocytes by promoting an increase in a thermogenic gene programme, appears to stimulate the expression of genes associated with beige fat thermogenesis and stimulate the immune cell subtypes to enter the adipose tissue, thus, activating pro-thermogenic actions (Rao et al., 2014). Increases in metabolic rate have been observed in obese, diabetic mice despite no changes in physical activity and food intake or any observed alterations in RER following adenovirus induced overexpression of Metrnl (Rao et al., 2014). The observed increases in EE were observed five days post-injection, suggesting the increase occurred in line with the thermogenic gene response adaptations and also suggests a longer term exposure to hypoxia may be necessary to evoke changes in circulating levels highlighting a future direction for investigation.

Interestingly a long term intermittent cold exposure in which mice were exposed to 4 °C, two hours a day, five days a week for 14 weeks induced an improved glucose tolerance, enhanced insulin sensitivity and reduced weights of epididymal and retroperitoneal adipose tissue in accordance with increased expressions of mitochondrial uncoupling protein (UCP1) and PGC1 α in subcutaneous adipose tissue (Wang et al., 2015b). These findings suggest that intermittent exposure to cold may improve glucose homeostasis and induce white adipose tissue “browning” thus serving as a possible intervention to metabolic disorders. An intermittent hypoxic programme may also therefore be of worth and should be considered as a future direction. Furthermore long term exposure is likely needed in order for browning of tissue to be observed. Work from Rao et al, (2014) also suggests Metrnl expression was seen to increase acutely in the triceps muscles 24 hours after a downhill running exercise bout. However the present study observed no measureable change in Metrnl concentration following acute hypoxia and moderate intensity exercise suggesting possible differences in the effects of exercise on response of Metrnl expression and concentration. Downhill

running and resistance exercise used in the study from Rao may therefore induce greater or differing mechanisms related to Metrnl expression compared to cycling exercise used in the present study and is a possible reason for observed differences. Furthermore as Metrnl release and its manufacture is determined by mRNA production its release may simply be due to more manufacture rather than any storage of the hormone. As such prolonged or repeated exposure to hypoxia may be necessary to expect an increase in Metrnl. Prolonged or repeated exposure to hypoxia may result in an increase in mRNA for Metrnl and thus increased transcription.

Technique of measurement may also contribute to the observed lack of change in measures in the current investigation. The analysis of blood plasma rather than skeletal muscle tissue analysis must also be considered as a potential reason for differences in findings in comparison to previous work (Rao et al. 2014). The severity of hypoxia should be highlighted as a potential factor for future investigation and a reason for lack of change. Furthermore although a rationale for an altitude induced change in Meteorin-like is presented it must be considered that the response to hypoxia may differ from that when exposed to conditions of cold. Moreover it is possible that hypoxia has the potential to oppose the effects of Meteorin-like, potentially through a blunting effect of the thermogenic response as has been observed when cold and conditions of hypoxia are combined (Blatteis and Lutherer. 1976; Gautier et al. 1991).

This study fits within the thesis by measuring the effects of an acute exposure of severe hypoxia previously explored in study 2 (Chapter 5) with the addition of moderate intensity exercise. This is situated prior to the investigation of a longer term altitude stay in study 4 (Chapter 7) and the post-altitude effects in study 5 (Chapter 8).

LIMITATIONS AND FUTURE DIRECTIONS

Data was collected on a healthy, recreationally active population in the current study in order to report the physiological response through alterations in EE and lipid metabolism from the protocol rather than to administer a weight loss intervention. Future work might focus on a target population of overweight or sedentary individuals in order to assess the response of the intervention on such groups, where weight management and loss of adiposity may be beneficial for health. Given that we observed little change with acute interventions; a longer term study, with repeated exposure and exercise bouts as a training intervention, would allow for the effectiveness of such a protocol to be determined for improvements in body mass and metabolism. As such study 4 (Chapter 7) in this thesis investigates the effect of an 18 day stay at an altitude of 3,400m on similar metabolic measures. Additional work is required in order to establish the acute effects of hypoxic exposure of differing lengths and extremities on Metrnl regulation as well as the effect of long term exposure to altitude, lasting for days or weeks. The current study observed responses to exercise at fixed relative exercise intensity; future work may also consider differing exercise types, frequency and intensity as well as incorporating intermittent exposures as possible interventions.

6.6 CONCLUSION

This study demonstrated that a short term hypoxic exposure at rest is sufficient in inducing increased EE through greater CHO and FAT oxidation. A predominance of CHO sources was observed following exposure to hypoxia and this was found both in the postprandial state and the fasted state. Moderate intensity exercise was seen to be useful for reducing postprandial hypertriglyceridaemia following a high fat meal. A 60 minute hypoxic exposure at rest induced increases in plasma FFA values although contrary to our hypothesis a prior exposure to hypoxia did not appear to alter EE or substrate use during a subsequent exercise bout. The main strength of the study is the novel approach of combining an acute hypoxic exposure with an exercise bout in normoxic conditions in an attempt to utilise the environmental stimulus as a tool for greater fat oxidation. Furthermore, despite similarities in the effects of cold and altitude/ hypoxia no previous investigations, to the author's knowledge, had assessed the response of MetrnI following exposure to hypoxia. Exposure to hypoxia, moderate intensity exercise and a HLD do not influence the acute regulation of MetrnI release in humans and this is the first study to show no acute effect of exercise, hypoxia or fat concentration on MetrnI.

7. MODERATE ALTITUDE INDUCES METABOLIC EFFECTS IN HEALTHY HUMANS ACUTELY AND FOLLOWING AN 18 DAY STAY AT 3,400M.

7.1 ABSTRACT

AIM: Mechanisms leading to altitude induced cachexia are poorly understood. The present study assessed the acute and intermediate term metabolic responses to moderate altitude. **METHOD:** Ten individuals (5 males, 5 females; age, 23 ± 4 yr.; BMI 24 ± 2 kg/m²) resided at 3,400m for 18 days. Body mass, composition and metabolic factors including, resting metabolic rate (RMR), substrate oxidation and taste thresholds were recorded prior to departure (PRE), at days 5, 12 and 18 (ALT5, ALT12 and ALT18) during stay at altitude and within one and four weeks post-return (POST25 and POST46). Meteorin-like (Metrnl) was measured at PRE, POST25 and POST46. **RESULTS:** Losses of body mass (BM) were recorded at ALT12 (-1.22 ± 0.98 kg), ALT18 (-2.36 ± 1.41 kg), ALT25 (-2.63 ± 1.31 kg), and POST46 (-1.89 ± 1.31 kg). Contribution to EE from carbohydrate (CHO) derived fuels increased at ALT18 (1430 ± 344 kcal.day⁻¹) compared to PRE (631 ± 295 kcal.day⁻¹). Greater fat use was observed at POST46 compared to ALT12 ($+ 0.05 \pm 0.03$ g.min⁻¹) and ALT18 ($+ 0.07 \pm 0.03$ g.min⁻¹) ($p < 0.05$). Sweet, salt and bitter taste sensations were reduced whilst at 3,400m ($p < 0.05$). No observed change Metrnl occurred post-return to sea level ($p > 0.05$). **CONCLUSIONS:** Lasting reductions in BM and greater contribution of CHO derived fuels at altitude agree with previous findings. Increased fat oxidation upon return to sea-level highlights the use of exposure to moderate altitude as a potential method for increased fat use and affecting body composition. This is the first study to show no lasting effect of short term moderate altitude exposure on Metrnl concentrations upon return to SL.

7.2 INTRODUCTION

A loss of body mass in humans following prolonged exposure to hypoxia is consistent and suggests a causal relationship between body mass change and hypoxic exposure (Boyer and Blume 1984; Butterfield 1996). An altitude of between 5,000m and 6,000m is regarded as the limit of energy balance for humans, above this energy intake is not sufficient for body weight maintenance (Westerterp 2001). Losses of between 5 – 15% of body mass are outlined at high altitude. Suggested contributing factors to weight loss at altitude include, amongst others, the conditions of the ascent, the final altitude attained and the duration of stay (Marriott and Carlson, 1996). Nevertheless knowledge of the mechanisms contributing to cachexia at altitude remains incomplete. Specifically, little is known regarding the induced alterations in potential markers of adipose tissue thermogenesis following a stay at moderate altitude in humans.

Increased EE and reduced energy intake may be significant contributors to loss of body mass at altitude (Westerterp 2001; Mawson et al. 2000; Lippl et al. 2010; Kayser and Verges 2013; Westerterp-plantenga et al. 2011; Westerterp and Kayser 2006). An increased expenditure of 500 kcal.day⁻¹ for men at 4,300m has been reported (Brooks, 2014), arising from an increased basal metabolic rate (BMR) and increased physical activity (Westerterp, 2001). Initial, immediately after ascent, increases in BMR of 27% have been reported in men at 4,300m and the metabolic rate has remained elevated at 17% above sea-level values, following three weeks of habituation (Butterfield et al., 1992). Similarly the effect of a seven day stay at 2650m, independent of exercise, induced lasting increases in metabolic rate of 3.4 kcal.kg⁻¹.day⁻¹ (Lippl et al., 2010) with an increase in sympathetic drive suggested as a cause (Palmer and Clegg 2014). Findings from previous chapters in this thesis (studies 2 and 3/ Chapters 5 and 6) have also highlighted increases in RMR upon acute exposure to severe hypoxia.

Alterations in the substrate contribution, i.e. fats, carbohydrate and protein, to EE are common at altitude (Azevedo et al. 1995; Braun 2008; Cartee et al. 1991; Young et al. 1982; Zinker et al. 1994). Specifically, hypoxia leads to greater dependence on glucose and less reliance on lipid in many experimental models including isolated muscle (Azevedo et al., 1995), whole rat models (Cartee et al., 1991) and exercising dogs (Zinker et al., 1994). The direct effect of altitude on substrate use however is problematic due to confounding factors present in the field including physical exertion, changes in environmental temperature, the length and extremity of exposure, altitude induced alterations in appetite, subsequent eating behaviour (Marriott and Carlson, 1996) and the energy balance of individuals (Marriott and Carlson, 1996). Nevertheless in men who are fed to meet energy needs carbohydrate, specifically glucose, is the main source of fuel at 4,300m following acclimatisation at rest and during exercise (Brooks et al., 1991). It is suggested CHO derived sources are more economical in an “oxygen-poor” environment, providing a greater yield of ATP per litre of oxygen consumed than fat (McClelland, 2004) which is seen in study 2 and 3 (Chapters 5 and 6) of this thesis.

Appetite suppression and reduced food intake has been reported at altitude in both field (Bailey et al., 2000; Barnholt et al., 2006; Kalson et al., 2010; Westerterp, 2001) and laboratory (Westerterp-Plantenga, 1999) studies. A reduced intake of ~ 200 kcal.day⁻¹ has been estimated from previous work at 4,300m (Butterfield et al., 1992), and reductions of between 20-40% in total energy and protein intake have been seen at higher altitudes (> 4,300m) (Rose et al. 1988; Kayser and Verges 2013). Decreased food availability and palatability at altitude are presumed contributors to the observed anorectic effect and cachexia (Palmer and Clegg 2014). Loss of body mass has been described as a direct consequence of reduced or lost sensations of taste (Woschnagg et al., 2002). Although the effect of altitude on taste has not been exhaustively examined some evidence does exist in this regard (Singh et al., 1997, 1996). Increases in taste thresholds for glucose and sodium chloride and decreases in taste thresholds for quinine sulphate and citric acid were observed in humans exposed to 3,500m for a three week period (Singh et al., 1997). These changes all showed a tendency to return to baseline upon return to sea-level suggesting an acute effect of altitude on taste and specifically that hypoxic stress increases the palatability for sweetness which may be caused by an anorexia linked stress. Similarly in a rat model the effect of a three week exposure to hypoxia corresponding to an altitude of 7,620m observed a preference for sweet tasting solutions during exposure that returned to pre-exposure levels upon restoration of normoxia (Singh et al., 1996). These latter findings highlight a potential alteration in food intake at high altitude based on an increased importance of sensory cues such as preference for sweet flavour. Cravings of sweet, salt, and bitter tastes have also been attenuated in humans during high altitude residency (2,616-4,200 m) (Yan et al., 2011) highlighting potential conflicting results. The observed reduction in craving however was present in high altitude natives rather than sea-level residents exposed to high altitude (Yan et al., 2011).

An increased monotony of flavour and thus, a reduced palatability and pleasantness of food culminating in earlier satiety and reduced energy intake are potential reasons for changes in taste resulting in altered body mass (Woschnagg et al., 2002). Accordingly the repeated presentation of some foods can lead to a persistent decrease in the pleasantness of the taste for those foods (Schutz and Pilgrim 1958; Siegel and Pilgrim 1958) and furthermore the consumption of a monotonous liquid diet, can result in voluntarily restriction of energy intake (Cabanac and Rabe 1976). Moreover, correlations exist between taste perception and anorexia, decreased food intake and weight loss (Ames et al., 1993; Dewys and Walters, 1975; Mattes and Cowart, 1994). Based on these previous findings information regarding the effects of altitude exposure on taste and EE, alongside changes in body composition and substrate use may improve our understanding of the mechanisms behind altered eating habits, metabolic changes and the reasons for altitude induced cachexia.

PGC-1 α is a transcriptional coactivator induced by exercise that controls the genes involved in oxidative metabolism and mitochondrial biogenesis (Ruas et al., 2012). A novel form of PGC-1 α has been identified (PGC-1 α 4), which is highly expressed in skeletal muscle, particularly during exercise in mice and humans (Ruas et al., 2012) highlighted by a 1.5 and a 3 fold increase in expression following

resistance exercise and combined endurance and resistance exercise respectively. Mice with skeletal muscle-specific transgenic over-expression of PGC-1 α 4 demonstrate muscle hypertrophy, increased basal EE and increased browning of white fat depots without changes in food intake or movement, which may contribute to the lean phenotype of these mice (Rao et al. 2014; Ruas et al. 2012). The expression of PGC-1 α 4 in skeletal muscle stimulates increased mRNA expression and secretion of the hormone Meteorin-like (Metrnl) (Rao et al., 2014). Meteorin-like protein has been identified as a key PGC-1 α 4 target gene which promotes thermogenesis. Meteorin-like protein is increased in mice following both a bout of concurrent (endurance and resistance exercise) and a bout of eccentric exercise (Rao et al., 2014). Meteorin-like protein expression was also increased following acute cold exposure (six hours at 4°C and 24 hours at 4°C) but not chronic exposure (two weeks at 4°C) (Rao et al., 2014). Given the importance of the relationship between Metrnl and metabolic changes that would drive fat use we hypothesised that the effects of a prolonged altitude stay would be to raise Metrnl concentrations and may provide a mechanism for altitude induced reduction in fat mass. This hypothesis is centred on the physiological similarities that exist upon exposure to both conditions of cold and hypoxia, namely an increased sympathetic nervous system activation.

The primary aims of the present study were; 1) to quantify the effects of an 18 day stay at 3,400m on BM, RMR, substrate use and appetite. 2) To quantify the effects of the sojourn on BM, RMR, substrate use and blood lipid measures upon return to sea-level. 3) To establish the effects of residence at 3,400m on three sensations of taste. 4) To observe the effects of prolonged hypoxia on Metrnl concentration upon return to SL. It was hypothesised that an 18 day stay at moderate altitude would induce increased RMR, acute reductions in appetite and taste sensation and lasting reductions in body mass. It was also hypothesised that Metrnl concentrations would increase in response to the altitude stay upon return to SL. This study fits within the thesis by investigating a prolonged altitude stay and its effects on the metabolic markers previously measured in the thesis in the acute setting.

7.3 MATERIALS AND METHODS

PARTICIPANTS

Ten individuals (5 males, 5 females) [23 \pm 4 years of age (mean \pm SD), body mass 73 \pm 11 kg, height 175 \pm 10 cm and BMI 24 \pm 2 kg/m²] agreed to partake in the study and complied with all criteria for participation [(3.5.1) Medical criteria and recruitment and (3.6) Pre-trial diet and exercise standardisation and (3.8) Hydration assessment. Prior to the undertaking of the experimental trials volunteers attended the laboratories whereby their anthropometric data was collected [(3.11) Anthropometric assessment].

EXPERIMENTAL DESIGN

The experimental design was split into sea level (SL) and altitude (AL) trials. The SL visits to the laboratory occurred within one week prior to travel to Peru (PRE) and on two occasions post-return

(POST25 and POST46). POST25 was conducted within one week of return. POST46 was conducted four weeks post-return.

The AL trials occurred on three separate occasions during the sojourn at 3,400m on days five (ALT5), 12 (ALT12) and 18 (ALT18). Participants travelled by air from London to Cusco (3,400m). Ambient temperature and relative humidity on the AL trials were 24.1°C and 36.5% at ALT5, 26.3°C and 30.2% at ALT12 and 22.2°C and 35% at ALT18. Participants were required to arrive between 6.00 and 7.00am. All SL trials were identical in their design as were AL trials with the addition of a taste test on the second AL visit. During the altitude stay participants were instructed to eat and drink ad libitum so as to reflect the effects of an altitude sojourn rather than to impose the effects of a strict diet. No restrictions were placed on physical activity during the altitude stay. Participants were instructed to maintain their normal level of activity as closely as possible.

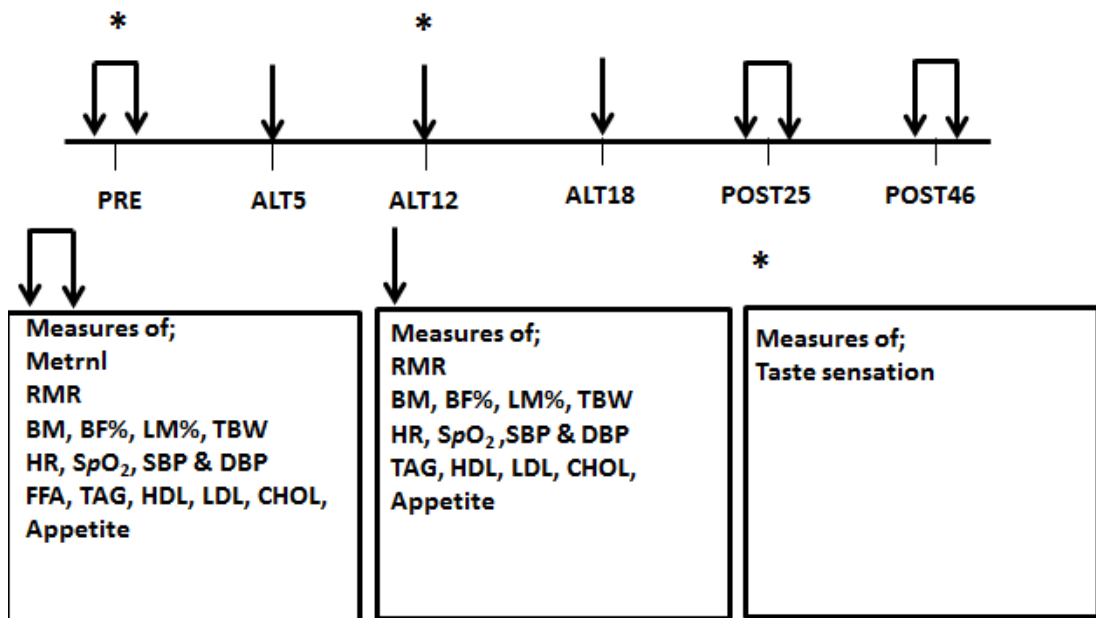


FIGURE 7.1: Schematic of study design.

Notes: Meteorin-like (Metrnl), resting metabolic rate (RMR), body mass (BM), body fat percentage (BF%), lean mass percentage (LM%), total body water (TBW), heart rate (HR), saturation of oxygen SpO₂, systolic blood pressure (SBP), diastolic blood pressure (DBP), free fatty acids (FFA), triglycerides (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol (CHOL).

EXPERIMENTAL MEASURES

A resting metabolic rate test (RMR) was completed during all trials at both SL and AL. Upon arrival participants were placed in a comfortable supine position in a quiet environment prior to recording metabolic data. Participants were instructed to lie down for a period of 45 minutes prior to the collection of all data during which individuals were habituated to the facemask. Following the rest

period participants were instructed to remain quiet and awake during the data collection. Expired air was measured using an online metabolic analyser (MetaMax3X, Cortex, Leipzig, Germany). Measures of $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER were taken during each RMR test. Female participants were tested throughout the menstrual cycle thus the use of contraceptive medication is likely. Heart rate (HR), peripheral arterial oxygen saturation (SpO_2) [(3.13.5) Heart rate and (3.13.4) Peripheral artery oxygen saturation] and systolic and diastolic blood pressure (SBP and DBP) were recorded during all trials at both SL and AL, following the 45 minute rest period. Blood pressure was measured using an automated blood pressure monitor (OmronM4, Omron Matsusaka Co. Ltd, Tokyo, Japan). The measurement was taken from the left arm on each occasion during which individuals were sat in an upright position following the rest period.

Substrate oxidation was determined indirectly via non-protein respiratory exchange ratio determined through expired gas for a 10 minute period at the end of the 45 minute rest assuming constant protein oxidation. Oxidation rates of fat and carbohydrate were calculated according to the equations of Peronnet and Massicotte (1991) [(3.13.3) Calculations derived from cardiopulmonary measures].

Heart rate and peripheral arterial oxygen saturation (SpO_2) were monitored using a HR monitor (Polar, Finland) and a fingertip pulse oximeter (Nonin 2500, Nonin Medical Inc., USA) respectively [(3.13.5) Heart rate and (3.13.4) Peripheral artery oxygen saturation]. TEE was calculated by assuming constant expenditure at each time point for 30 min.

Measures of body mass (BM) were collected to the nearest 10g using portable, calibrated weighing scales (Adam GFK 150) in the morning prior to any food intake and after voiding. The same scales were used across trials. Assessment of body fat percentage (BF %), lean mass (LM %) and total body water (TBW) was conducted using a Quadscan multi-frequency bioelectrical impedance device (BIA) (Bodystat, Isle of Man), a valid method of estimating body fat when individuals are within normal body fat range (Sun et al., 2005). Measures of body composition were conducted during all trials at both SL and AL at the same time points as RMR collection.

Subjective feelings of appetite were reported on paper using a 100mm visual analogue scale (VAS) (Flint et al., 2000). The scale was anchored by answers (“Not at all hungry” vs “As hungry as I have ever felt”) to the question (“How hungry do you feel”) posed above the line. Such scales have been shown to have good reproducibility and are deemed reliable for appetite research (Flint et al., 2000). Appetite was measured at altitude at PRE, ALT5, ALT12, ALT18, POST25, POST46 time points.

Experimental design and measures taken are illustrated in Figure 7.1.

During altitude exposure a modified Lake Louise Questionnaire (LLQ) (Roach et al., 1993) was used to monitor symptoms of AMS every morning upon awakening [(3.14.1) Lake Louis Questionnaire score].

BLOOD MARKERS

Fasted venous blood samples were taken during the SL trials (PRE, POST25 and POST46) [(3.17.1) Cannulation]. With plasma separated [(3.17.2) Blood plasma separation] FFA and MetrnI were analysed [(3.17.3) Analysis of FFA and (3.17.5) Analysis of Meteorin-like].

Fasted fingertip blood samples (AccucheK Softclix Pro, Roche, Lewes, England) were collected at all-time points throughout the SL and AL trials for the analysis of the participant's lipid profile using the CardioChek PA Meter kit (CardioChek, USA). Values from the lipid profile included those of triglycerides (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL), and cholesterol (CHOL).

TASTE TEST

A taste test was conducted on two occasions throughout the testing period following a familiarisation trial. The initial test was conducted within one week of departure to Peru (PRE) with the second completed on day 12 of the sojourn (ALT12). Participants were tested for three taste sensations; sweet, salt and bitter. Six aqueous solutions of differing concentrations were made for the sweet sensation and five solutions were made for both the salt and bitter sensations. The differing concentrations of sucrose comprising the sweet set were 0, 1, 1.5, 3, 6 and 12 g per 100ml. Salt tastes were comprised of concentrations of sodium chloride of 0, 0.06, 0.09, 0.18, 0.36 g per 100ml and bitter comprised of citric acid concentrations of 0, 0.1, 0.2, 0.3, 0.4 g per 100ml. All concentrations were supplied in 100ml servings.

Participants were given the concentrations in a randomised order. Participants were told to hold the solution in their mouth for 3 seconds before disposing of the sample. Between each differing concentration participants were instructed to rinse their mouth with plain water. Participants were instructed to state whether they could taste "something" or "nothing", other than water, with each sample. All solutions were free from colour and mixed in order to blind the participants. Concentrations of each taste sensation were converted to a "taste point" value in line with the order within the sequence in which they were presented to the participant. Similar methods have previously been employed for measuring preference for sweet taste (Asao et al. 2012; Liem and Degraaf 2004; Mennella et al. 2011) and found to be reproducible demonstrated through between day intraclass correlation coefficients (Asao et al., 2012).

STATISTICAL ANALYSIS

All data were checked for normality and sphericity [(3.15.2.1) Normal distribution and (3.15.2.2) Sphericity] and were adjusted using the Huynh-Feldt method. All data were analysed using a standard statistical package (SPSS version 20 for Windows 7).

One-way analysis of variance (ANOVA) with repeated measures were carried out over three time points (PRE, POST25 and POST46) for all blood measures of MetrnI and FFA. One-way ANOVA with repeated measures was carried out over six time points (PRE, ALT5, ALT12, ALT18, POST25 and POST46) for measures of BM, BMI, BF%, LM %, TBW, SBP, DBP, EE, substrate partitioning, TAG, HDL, LDL, CHOL. Measures of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, and RER were also analysed over six time points. Bonferoni-pairwise comparisons were used to compare between separate time points throughout the testing time frame. Effect sizes reported are Cohen's d and partial eta squared (ηp^2). The recommendations for these calculations are based upon the review of Lakens (2013).

A Wilcoxon statistical analysis was carried out on the "Taste Point" value for the taste test trials. Pearson's correlation coefficient was conducted on measures of SpO_2 and BM, RMR and BF% to determine extent of correlation. Significance was set at $p < 0.05$ for all analysis. All data were analysed using a standard statistical package (SPSS version 20).

7.4 RESULTS

PHYSIOLOGICAL MEASURES

Changes in BM (kg) were observed throughout the investigation ($F_{(5, 40)} = 10.329$, $p = 0.002$, $\eta p^2 = 0.564$). Significant losses of BM were observed compared to PRE at ALT12 ($p = 0.015$, $d = 0.22$), ALT18 ($p = 0.005$, $d = 0.25$), POST25 ($p = 0.001$, $d = 0.2$) and POST46 ($p = 0.037$, $d = 0.17$) (Figure 7.2) equating to, 3.2 ± 1.8 , 3.6 ± 1.5 , 3.0 ± 1.1 and $2.5 \pm 1.8\%$ reductions in BM respectively. Compared to ALT5 losses in body mass were also observed at ALT18 ($-1.41 \pm 0.9\text{kg}$, $p = 0.023$, $d = 0.14$) and POST25 ($-1.0 \pm 0.69\text{kg}$, $p = 0.037$, $d = 0.09$) equating to 1.9 ± 1.1 and $1.4 \pm 0.9\%$ reductions in BM respectively. Male and female data for changes in BM and relative to body mass EE are presented in Figure 7.3. No differences in the change between sexes was observed for body mass ($F_{(5, 40)} = 1.900$, $p = 0.119$, $\eta p^2 = 0.213$) or relative RMR ($F_{(5, 40)} = 1.092$, $p = 0.062$, $\eta p^2 = 0.250$).

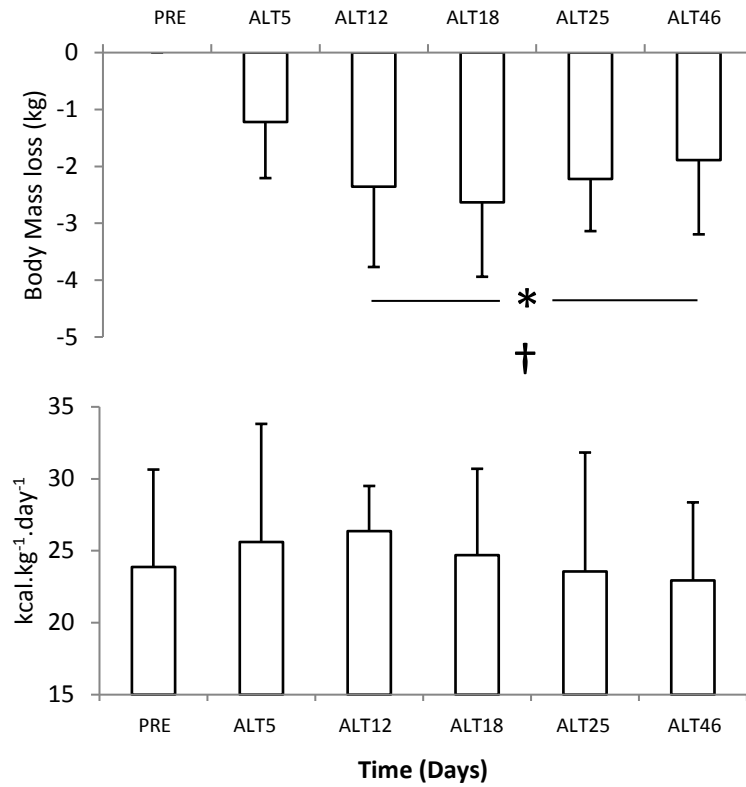


FIGURE 7.2: Body Mass loss and resting metabolic rate in kcal.kg⁻¹day⁻¹ from PRE to DAY46.

Notes: * Illustrates difference compared to PRE measures. † illustrates differences compared to DAY5.

Resting metabolic rate (RMR). All significance symbols correspond to $p < 0.05$.

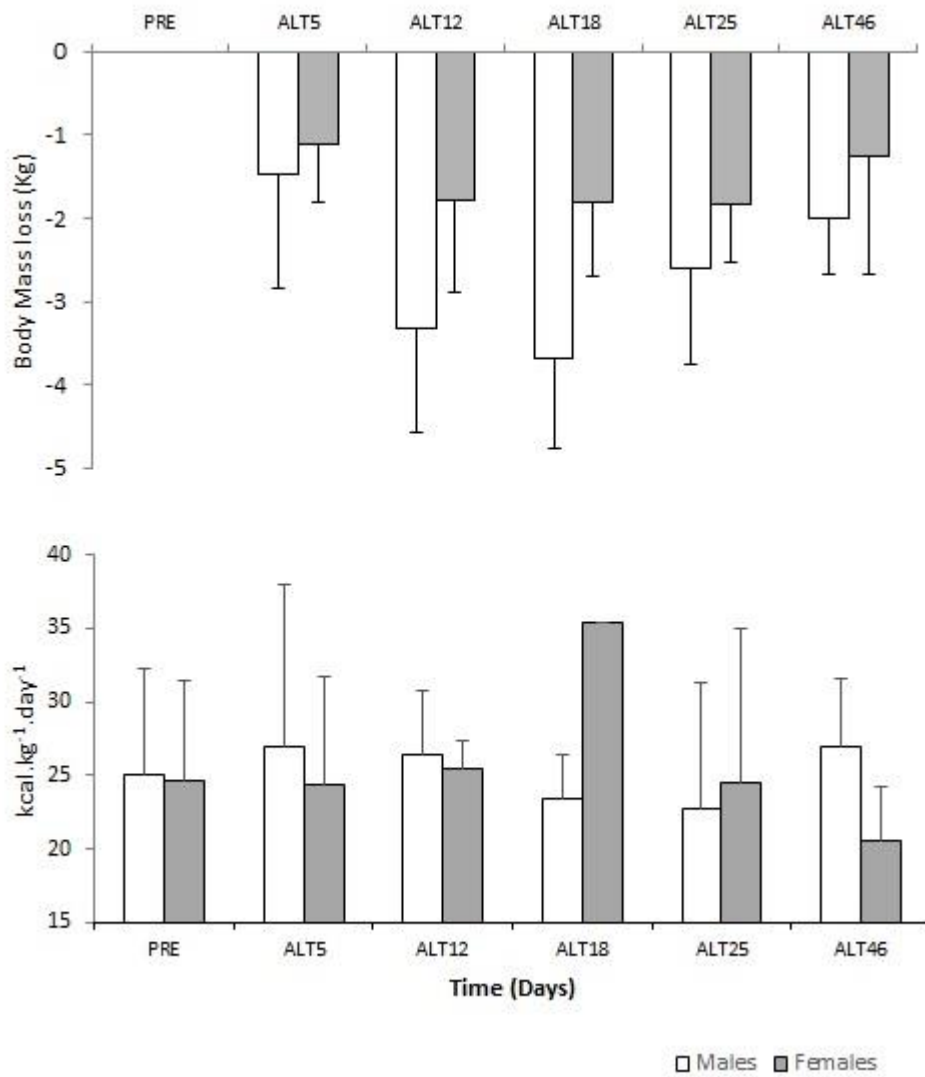


FIGURE 7.3: Body Mass loss (kg) and relative resting metabolic rate ($\text{kcal.kg}^{-1}\text{day}^{-1}$) from PRE to DAY46 for males and females separately.

No changes in BF% ($F_{(5,40)} = 1.427, p = 0.263, \text{np}^2 = 0.169$), LM% ($F_{(5,40)} = 1.309, p = 0.295, \text{np}^2 = 0.141$) or TBW ($F_{(5,40)} = .775, p = 0.521, \text{np}^2 = 0.100$) were recorded throughout the testing period suggesting body mass loss was shared amongst compartments (Table 7.1).

Table 7.1: Measures of body composition, metabolic rate and substrate use from PRE to POST46. BM, Body mass. BMI, Body Mass Index. BF%, Body fat percentage. LM%, Lean mass percentage. TBW%, Total Body Water percentage. RMR, Resting metabolic rate. CHO, Carbohydrate oxidation. FAT, Fat oxidation. RER, Respiratory Exchange Ratio. * Illustrates significant differences compared to PRE; † illustrates significant differences compared to ALT5; # illustrates significant differences compared to ALT12; α illustrates significant differences compared to ALT18. All significance symbols correspond to $p < 0.05$.

	PRE	ALT5	ALT12	ALT18	POST25	POST46
BM (kg)	72.9 ± 11.3	71.7 ± 11.2	70.5 ± 10.9*†	70.2 ± 10.6*†	70.7 ± 10.8*†	71 ± 10.8*
BMI (kg/m²)	23.6 ± 1.6	23.2 ± 1.7	22.8 ± 1.7*†	22.7 ± 1.6*†	22.9 ± 1.6*†	23 ± 1.6*
BF (%)	18.7 ± 7.6	18.4 ± 7.2	19.4 ± 8	19.0 ± 5.9	18.6 ± 6.8	19.2 ± 7.4
LM (%)	80.8 ± 7.3	81.9 ± 6.8	79.0 ± 8.5	80.6 ± 5.6	79.5 ± 7.2	80.1 ± 6.9
TBW (%)	55.9 ± 6.0	56.8 ± 5.6	56.9 ± 9.7	54.4 ± 4.4	56.1 ± 5.1	55.4 ± 5.8
RMR (kcal.min⁻¹)	1.2 ± 0.3	1.3 ± 0.4	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.4	1.1 ± 0.4
CHO (g.min⁻¹)	0.40 ± 0.09	0.41 ± 0.16	0.43 ± 0.11	0.33 ± 0.11	0.37 ± 0.10	0.40 ± 0.06
FAT (g.min⁻¹)	0.07 ± 0.04	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.08 ± 0.05	0.08 ± 0.03#α
RER	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
HR (b.min⁻¹)	70 ± 8	81 ± 20	58 ± 10	55 ± 15		
SpO₂ (%)	98 ± 1	91 ± 3	93 ± 2	93 ± 3		

No differences in resting $\dot{V}O_2$ ($F_{(5, 40)} = 0.862$, $p = 0.515$, $np^2 = 0.97$), or $\dot{V}CO_2$ ($F_{(5, 40)} = 2.130$, $p = 0.082$, $np^2 = 0.210$) were observed throughout the testing period. Greater RER values ($F_{(5, 40)} = 5.571$, $p = 0.001$, $np^2 = 0.410$) were observed at ALT12 ($p = 0.019$, $d = 1$) and ALT18 ($p = 0.003$, $d = 1$) compared to POST46. Alterations in HR were observed throughout the testing period ($F_{(5, 40)} = 4.001$, $p = 0.019$, $np^2 = 0.333$) characterised by an increase at ALT5 compared to PRE ($p = 0.03$, $d = 1.2$) followed by a subsequent return to SL values. Heart rate values at ALT12 of 58 ± 10 b.min⁻¹ ($p = 0.02$, $d = 0.5$) and ALT18 of 55 ± 15 b.min⁻¹ ($p = 0.006$, $d = 1.5$) were lower than the values of 81 ± 20 b.min⁻¹ at ALT5.

Decreases in SpO_2 ($F_{(5, 40)} = 54.588$, $p < 0.001$, $np^2 = 0.661$) occurred at each altitude testing day (ALT5, 12 and 18) ($p \leq 0.05$) compared to PRE. PRE values of $98 \pm 1\%$ reduced to $91 \pm 3\%$ ($p < 0.01$, $d = 3.13$) $93 \pm 2\%$ ($p < 0.01$, $d = 3.16$) and $93 \pm 3\%$ ($p < 0.01$, $d = 2.24$) at ALT5, ALT12 and ALT18 respectively. Significant negative correlations were observed between the change in body mass at ALT18 from PRE values with changes in SpO_2 during the same time period ($r^2 = 0.6$, $r = -0.8$, $p = 0.01$) and BF% ($r^2 = 0.5$, $r = -0.7$, $p = 0.03$). Measures of LLQ were unchanged throughout the investigation ($p = 0.089$, $np^2 = 0.637$).

No changes in systolic ($F_{(5, 40)} = 1.254$, $p = 0.312$, $np^2 = 0.136$) or diastolic ($F_{(5, 40)} = 2.102$, $p = 0.126$, $np^2 = 0.208$) blood pressure were observed throughout the testing period. Mean systolic values of 131 ± 13 mmHg were observed at PRE, 123 ± 14 mmHg at DAY18 following altitude stay and 131 ± 18 mmHg at DAY46 four weeks post-return to SL.

ENERGY EXPENDITURE AND SUBSTRATE UTILISATION

RMR remained similar throughout the testing period ($F_{(5, 40)} = 1.091$, $p = 0.390$, $np^2 = 0.179$). Individuals expended 23.9 ± 6.8 kcal.kg⁻¹.day⁻¹ prior to the sojourn, 24.7 ± 6.0 kcal.kg⁻¹.day⁻¹ at ALT18 following the stay and 22.9 ± 5.4 kcal.kg⁻¹.day⁻¹ four weeks post-return to SL (Figure 7.2). A significant negative correlation between SpO_2 and RMR (kcal.min⁻¹) ($r^2 = 0.5$, $r = -0.7$, $p = 0.03$) was observed at ALT18.

An increase in CHO contribution towards EE was observed during the altitude stay compared to SL values ($F_{(5, 40)} = 9.017$, $p < 0.001$, $np^2 = 0.530$). Greater CHO contribution was recorded at ALT18 compared to PRE ($p = 0.045$, $d = 2.49$) so that 1430 ± 344 kcal.day⁻¹ were derived from CHO sources at ALT18 compared to 631 ± 295 kcal.day⁻¹ at PRE. This equated to $79 \pm 15\%$ at ALT18 compared to $38 \pm 17\%$ at PRE of total expended energy ($p \leq 0.05$). Lower FAT utilisation occurred at these corresponding time points so that at PRE individuals were expending 1082 ± 440 kcal.day⁻¹ ($62 \pm 17\%$ contribution) derived from FAT compared to 376 ± 266 kcal.day⁻¹ ($21 \pm 15\%$ contribution) at ALT18.

Greater FAT contribution was recorded upon return to SL at POST46 compared to that at ALT12 ($p = 0.019$, $d = 1.41$) and ALT18 ($p = 0.003$, $d = 2.04$) corresponding to lower CHO contribution ($p \leq 0.05$). FAT derived fuels were contributing to 1138 ± 455 kcal.day⁻¹ which equated to $71 \pm 21\%$ of total expended calories at POST46 in comparison to 567 ± 348 kcal.day⁻¹ at ALT12 equating to $29 \pm 14\%$ of total calories and 376 ± 266 kcal.day⁻¹ at ALT18 equating to $21 \pm 15\%$ of total calories (Figure 7.4).

Greater oxidation of fat ($p < 0.001$, $np^2 = 0.422$) occurred at POST46 compared to ALT12 ($p = 0.048$, $d = 1.57$) and ALT18 ($p = 0.005$, $d = 2.35$). Individuals oxidised $0.08 \pm 0.03 \text{ g}\cdot\text{min}^{-1}$ at DAY46 compared to $0.04 \pm 0.02 \text{ g}\cdot\text{min}^{-1}$ at ALT12 and $0.02 \pm 0.02 \text{ g}\cdot\text{min}^{-1}$ at ALT18. Despite greater contribution to total expended energy whilst at 3,400m CHO oxidation remained stable over time ($p = 0.227$, $np^2 = 0.154$). Individuals were utilising $0.40 \pm 0.09 \text{ g}\cdot\text{min}^{-1}$ at PRE, $0.33 \pm 0.11 \text{ g}\cdot\text{min}^{-1}$ at ALT18 and $0.40 \pm 0.06 \text{ g}\cdot\text{min}^{-1}$ at POST46 (Table 7.1).

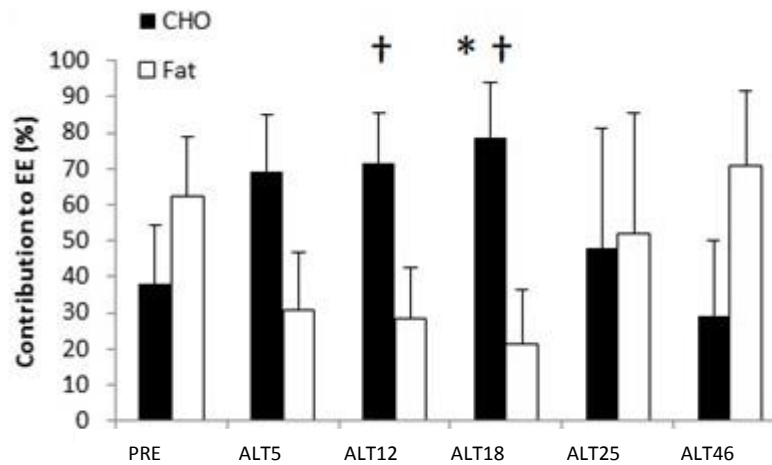


FIGURE 7.4: The contribution of carbohydrate and FAT (%) to resting energy expenditure.

Notes: Carbohydrate (CHO). * Illustrates greater CHO contribution compared to PRE measures. † illustrates lower FAT contribution compared to DAY46. All significance symbols correspond to $p < 0.05$.

BLOOD ANALYSES

No alterations in the concentrations of FFA ($F_{(4, 32)} = 1.009$, $p = 0.438$, $np^2 = 0.201$) and TAG ($F_{(4, 32)} = 2.937$, $p = 0.083$, $np^2 = 0.269$) were observed throughout the investigation. Similarly measures of CHOL ($F_{(5, 40)} = 0.939$, $p = 0.466$, $np^2 = 0.105$), HDL ($F_{(5, 40)} = 1.102$, $p = 0.380$, $np^2 = 0.155$), and LDL ($F_{(5, 40)} = 1.121$, $p = 0.466$, $np^2 = 0.105$) concentrations remained similar throughout (Table 7.2).

Measures of Metrnl were unchanged over time ($F_{(2, 12)} = 1.092$, $p = 3.67$, $np^2 = 0.154$). Concentrations of $0.31 \pm 0.10 \text{ ng/mL}$ at PRE, $0.27 \pm 0.10 \text{ ng/mL}$ at POST25 and $0.31 \pm 0.06 \text{ ng/mL}$ at DAY46 were observed.

Table 7.2: Measures of blood pressure and blood lipids from PRE to DAY46. SBP, Systolic blood pressure. DBP, Diastolic blood pressure. FFA, Free fatty acids. TAG, Triglycerides. CHOL, Cholesterol. HDL, High density lipoprotein. LDL, Low-density lipoprotein. SBP, Systolic blood pressure. DBP, Diastolic blood pressure. FFA, Free fatty acids. TAG, Triglycerides. CHOL, Cholesterol. HDL, High density lipoprotein. LDL, Low-density lipoprotein.

	PRE	ALT5	ALT12	ALT18	POST25	POST46
SBP (mmHg)	131 ± 13	133 ± 22	127 ± 19	123 ± 14	129 ± 16	131 ± 18
DBP (mmHg)	76 ± 8	82 ± 13	78 ± 11	84 ± 11	79 ± 9	81 ± 11
FFA (mmol.L⁻¹)	0.34 ± 0.04				0.32 ± 0.07	0.29 ± 0.03
TAG (mg.dL⁻¹)	124 ± 29.51	97.43 ± 35.43	106.28 ± 14.89	123.9 ± 18.4	100.68 ± 32.14	115.14 ± 13.15
CHOL (mmol/L)	3.3 ± 0.3	3.3 ± 0.5	3.5 ± 0.8	3.7 ± 0.8	3.5 ± 0.8	3.3 ± 0.2
HDL (mmol/L)	1.2 ± 0.1	1.3 ± 0.5	1.3 ± 0.6	1.2 ± 0.3	1.3 ± 0.5	1.4 ± 0.5
LDL (mmol/L)	1.6 ± 0.4	1.2 ± 0.2	2.1 ± 0.6	2.2 ± 0.6	1.8 ± 0.9	1.7 ± 0.5

MARKERS OF APPETITE AND TASTE

No changes in appetite ($F_{(5, 40)} = 5.039, p = 0.125, \eta^2 = 0.386$) or stomach sensation ($F_{(5, 40)} = 1.849, p = 0.165, \eta^2 = 0.188$) were observed throughout the observation period.

Taste sensation was reduced at altitude for sweet ($T = 28, p = 0.015$), salt ($T = 32, p = 0.046$) and bitter ($T = 45, p = 0.005$) compared to PRE so that the “first taste” in each flavour occurred at greater concentrations of flavour when at altitude. Values equating to a 115 ± 88 , 178 ± 177 , and $122 \pm 44\%$ increase in sweet, salt and bitter concentration prior to the first taste occurred at altitude respectively suggesting a 15% increase in sweet flavour, a 78% increase in salt flavour and a 22% increase in sour flavour is required at 3,400m to elicit the same taste as at SL.

Table 7.3: Measures of Appetite, stomach sensation, and Lake Louise Questionnaire.

	PRE	ALT5	ALT12	ALT18	POST25	POST46
APPETITE (%)	38 ± 29	30± 32	55 ± 30	43 ± 34		
STOMACH (%)	12 ± 17	3 ± 14	10 ± 10	7 ± 5		
LLQ	1 ± 1	4 ± 4	1 ± 1	1 ± 2		

7.5 DISCUSSION

Knowledge of the mechanisms contributing to cachexia at altitude is incomplete. Our study extends previous findings by examining the lasting effect of prolonged hypoxia on substrate use, body mass and notably MetrnI concentrations. These data show lasting reductions in body mass, a shift towards greater CHO use during a period of living at altitude and a reduction in taste sensation. Contrary to our hypothesis no significant changes in MetrnI, upon return to sea-level, or resting metabolic rate throughout were recorded and it seems unlikely that the browning of fat is a major mechanism accounting for the observed weight losses.

BODY MASS AND RESTING METABOLIC RATE

Losses of body mass following an 18 day stay at 3,400m were similar to outlined reductions in a review (Marriott and Carlson, 1996) suggesting a loss of 150 g/day is typical. Mean losses of 146 g/day on average at ALT18 in the present investigation reflect this similarity with previous findings including a study from Macdonald et al., (2009) who observed losses of 110g/day at an altitude ranging from 900m to 5,400m for 21 days. Similarly to work from Mawson et al. (2000) the greatest rate of change in body mass occurred within the first five days of exposure, with an average decrease of 1.2kg occurring in our study. Postulated mechanisms for losses of body mass at altitude include higher metabolic rate, greater energy output, a reduced feeling of hunger coupled with decreased food intake, reduction of intestinal energy uptake (> 5,500m) (Butterfield et al., 1992) and several endocrine factors (Kayser and Verges 2013). In our study there was no measurable change in appetite, indicating that this is not a major factor in energy deficit leading to weight loss

Losses of body mass in the present investigation were seemingly not compartmentalised to a greater ratio of fat or muscle demonstrated by the analysis of TBW, BF and LM through multi-frequency impedance. Previous findings regarding body-composition changes at altitude are inconsistent (Kayser 1992; Butterfield 1996; Tanner and Stager 1998). Initial loss of body mass at altitude has previously been attributed, in part, to losses of body water (Lippl et al., 2010) followed by a subsequent loss of fat mass and muscle wasting (Kayser, 1992) which cannot be excluded in the current study. The proportion of weight loss from each compartment is debated and dependent on numerous factors including, amongst others, the level of ascent, time spent at altitude, and the measurement technique (Wing-Gaia, 2014). As such previous investigations have observed loss of body mass derived from greater proportions of fat mass following exposure to high altitude (2,200 – 4,300m) (Tanner and Stager 1998) and from greater proportions of fat-free mass, at altitude (Macdonald et al., 2009) and in chamber studies (Krzywicki et al., 1969; Rose et al., 1988). Lack of change in body composition measures in the present study therefore suggest a loss of body mass that encompassed a combination of losses from fat and fat-free mass.

Contrary to previous investigations and thesis chapters (study 2 and 3/ Chapters 5 and 6), which have reported increases in resting metabolic rate (Butterfield et al., 1992; Lippl et al., 2010; Mawson et al., 2000) at altitude, no changes could be measured in the present study. Although not fully understood, suggested mechanisms for increased metabolic rate include an increase in sympathetic drive and thyroid activity (Hamad and Travis, 2006; Mawson et al., 2000). Early studies observed that an initial elevation in EE at rest declined to near sea-level by two to three weeks of exposure (Hannon and Sudman 1973). Work from Butterfield et al. (1992) on seven males reported a 27% increase in BMR on the second day of a three-week stay at 4,300m, which subsequently reduced to a 17% increase compared to sea-level values by day 10. Similarly, exposure to 4,300m of sixteen adequately fed healthy women induced a small and transient 6.9% increase in BMR after three days, which subsequently returned to sea-level values after six days (Mawson et al., 2000). Based on this work from Mawson et al. (2000) it is possible therefore, that the first evaluation of RMR occurring at ALT5 in the current study may have resulted in an initial rise in metabolic rate being missed before EE subsiding to SL values. A transient rise in metabolic rate has previously been described elsewhere (Hannon and Sudman 1973) supporting this suggestion.

Individual RMR responses varied considerably in the present investigation from PRE to ALT18 highlighted by a range from a 49% increase to a 25% decrease. This variation, coupled with residence at a lower altitude in the present study in comparison to previous literature may in part explain the lack of change in metabolic rate. Fitness levels of the participants may also play a role as previous literature has shown a smaller increase in BMR in individuals classified as “fit” ($VO_2 \text{ max} > 42 \text{ ml.kg.min}^{-1}$) compared to “unfit” ($VO_2 \text{ max} < 42 \text{ ml.kg.min}^{-1}$) (Mawson et al., 2000). Although $VO_2 \text{ max}$ data was not collected in the current study the recreationally active nature and age of the participants used suggest that this may be a plausible explanation for lack of change in RMR. As previously outlined, differences in the response to altitude on metabolic rate exist between males and females and in females throughout different stages of the menstrual cycle (Brooks, 2014; Solomon et al., 1982). In the current study males consistently had a higher absolute EE ($1.36 \pm 0.27 \text{ kcal.min}^{-1}$ and $1.06 \pm 0.26 \text{ kcal.min}^{-1}$) yet no differences between relative EE values ($25.27 \pm 6.42 \text{ kcal.kg}^{-1}.\text{day}^{-1}$ and $23.79 \pm 6.37 \text{ kcal.kg}^{-1}.\text{day}^{-1}$). No change over the course of the testing time frame were seen between sexes suggesting the lack of change in RMR was not due to the mixed sex participant group. Nevertheless altered metabolic rate amongst females throughout the different stages of the menstrual cycle likely occurred during the investigation thus resulting in an increased variation of results. This potential variation must be considered throughout the interpretation of results as changes in this measure cannot be attributed to the effect of altitude alone.

Reductions in energy intake have previously been reported at altitude in studies in conjunction with reduced body mass (Lippl et al., 2010) with suggestions that energy intake is the dominant determinant of body mass loss during prolonged exposure (Kayser and Verges 2013). A review by Butterfield (Marriott and Carlson, 1996) found that a reduction of roughly $200 \text{ kcal.day}^{-1}$ accounted

for the effect, whereas in obese individuals at 2,650m for seven days led to greater reductions in energy intake, of 734 kcal.day⁻¹, despite food being readily available (Lippl et al., 2010). Furthermore reductions in the perception of hunger (Kayser, 1992; Tschöp et al., 1998) and earlier feelings of satiety (Westerterp et al., 1985) have also been measured. The lack of significant change in self-reported appetite within an environment in which food was unrestricted and readily available most likely, the authors suggests, translated to unchanged eating habits in the present study. This however must be viewed with caution as no comprehensive energy intake measures were applied.

Individuals exposed to high altitude may have to adapt to both the hypoxemic effects of altitude and the metabolic effects of a negative energy balance simultaneously. In an attempt to reduce basal energy needs during a period of negative energy balance it has been proposed that the body tends to blunt some of the same neuroendocrine systems that are stimulated by hypoxia (Barnholt et al., 2006). As such, energy deficit and a reduction in sympathetic effect may explain the lack of observed change in RMR in the present study. A loss of metabolically active tissue and body mass as a consequence of a negative energy balance contributes to reduced EE as an interaction between energy balance and metabolic rate exists (Marriott and Carlson, 1996). Accordingly the maintenance of energy balance through enforced feeding and the matching of energy intake with estimated energy need at altitude has been previously shown, in some cases, to increase BMR and minimise loss of body mass (Roberts et al. 1996).

Previous literature regarding the effect of energy balance on changes in body composition at altitude is however contradictory. Work from Macdonald et al., (2009) observed that an increased energy intake failed to prevent loss of both fat and fat-free mass in individuals during a typical high altitude expedition despite an increase of between 10,000 – 15,000 kcal over the 21 days through the consumption of CHO drinks, which met the increased estimated energy demands induced by the hypoxia per se in more than half of the participants. These latter findings suggest that a primary role for negative energy balance leading to changes in body composition at altitude is not the most important factor and indicate that other contributory factors are present. Greater reductions in oxygen saturation were correlated to greater reductions in body mass from PRE to ALT18 in the present study. Reduced *SpO₂* may suggest a more pronounced response to altitude. Such a response may highlight an increase in physiological stress which when viewed in accordance with a correlation between reduced *SpO₂* and increased RMR in the present study may suggest a link between *SpO₂* and greater losses of body mass at moderate altitude through raised metabolic rate. Alternatively reduced *SpO₂* may illustrate an appropriate response to altitude highlighting an attempt to maintain body pH and supply sufficient oxygen to the tissues at the cost of a small drop in saturation in an environment of reduced oxygen availability. Such a response may be seen as an adaptive response to exposure to altitude. Further work however is required in order to elucidate the effects of *SpO₂* at moderate altitude and its role in factors leading to loss of body mass.

APPETITE AND TASTE SENSATION

Previously a decrease in pleasantness of taste has occurred in conjunction with reduced appetite and an increase in satiety at high altitude (5,000m) (Westerterp-Plantenga, 1999). Changes in AMS symptoms have also contributed to alterations in appetite (Lippl et al, 2010) however a reduced appetite has been maintained despite the return of AMS symptoms to baseline (Westerterp-Plantenga, 1999) suggesting AMS symptoms may be transient contributors to alterations in eating habits. In support of this suggestion a significant increase in AMS symptoms at ALT5 in the present study did not translate to alterations in self-reported appetite. Furthermore no correlation between AMS and appetite values was observed throughout the altitude stay (results not shown).

At ALT12 the initial increase in symptoms of AMS reduced to baseline levels in conjunction with reductions in sensations of taste. These findings suggest that alterations in taste sensation can occur or are maintained independently of symptoms of AMS. In the constancy of self-reported appetite and hunger, it is most likely that alterations in sensation of taste had little or no effect on hunger or appetite within an environment in which food was unrestricted and readily available. The role of taste and its effect on eating habits at altitudes of various severities however warrants further investigation. Although scores of appetite and hunger in the current study were not associated with AMS, further work is also required to explore this mechanism. The low occurrence of AMS in the current study may obscure the true effect of severe AMS on food intake at altitude. Reductions in sensation of taste may translate to a reduction in pleasantness of taste and alter food preferences ultimately culminating in reduced intake. Further research is required to confirm reductions in taste sensation and to determine the practical implications of such changes.

Environmental temperature has been shown to alter energy requirements with evidence suggesting that both a cold (Kojima et al., 2015) and a hot environment may suppress appetite (Hill et al., 2015). An ambient temperature in the current study of $24.4 \pm 2.6^{\circ}\text{C}$ suggests that temperature is unlikely to have been a contributing factor to any changes in appetite and subsequent eating habits. Nevertheless although not considered high, these temperatures are greater than experienced in the UK at a similar time of year and cannot be completely disregarded as an attributable factor to alterations in appetite and subsequent eating behaviour.

SUBSTRATE UTILISATION

Increases in CHO contribution to resting metabolism, in the current investigation, were observed with an expenditure of $1430 \pm 344 \text{ kcal}\cdot\text{day}^{-1}$ derived from CHO at ALT18 compared to $631 \pm 295 \text{ kcal}\cdot\text{day}^{-1}$ at PRE. These findings, coupled with increased RER values, agree with findings from thesis study 2 and 3 (Chapters 5 and 6) and with the frequently reported shift in substrate use at altitude to favour greater carbohydrate utilisation, upon acute and chronic exposure in previous literature (Brooks et al. 1991; Braun et al. 2000; Roberts, 1996). Such findings are generally attributed to an "oxygen saving

effect" of glucose utilisation (McClelland et al., 1998) with hypoxic inducible factor recently indicated as an important mediator in the metabolism alterations (Palmer and Clegg 2014). An up-regulation of the transcription factor HIF leads to a greater dependency on glucose uptake as an adaptive response to altitude when oxygen availability is limited (Palmer and Clegg 2014) with such increases observed after seven to nine days albeit at a higher altitude of 4,559m (Robach et al., 2007).

Changes in substrate utilisation at altitude are also affected by alterations in energy balance (Brooks, 2014; Marriott and Carlson, 1996). As such it is often difficult to separate the effects of hypoxia per se and the effect of negative energy balance on substrate use. Whilst fat is largely utilised for energy by the rest of the body and is predominant during times of non-exercise It is suggested that when energy needs are met by diet there is little utilisation of lipid sources by working muscles at altitude as measured by indirect calorimetry, stable isotope tracers and mass balance measurements for metabolites and tracers (Brooks, 2014). Based on this suggestion an increase in CHO contribution in the present study may point towards adequate energy intake although this should be interpreted with caution with the current data. Nevertheless these findings coupled with a reduction in body mass suggest alternative reasons to negative energy balance may exist for loss of mass at altitude as supported by previous work (Macdonald et al., 2009).

Previous findings have reported an increase in the utilisation of fat sources immediately after acute exposure to hypoxia (Workman and Basset 2012). These findings are suggested to be as a result of the repayment of an oxygen deficit caused by a reduced FiO_2 resulting in an increased autonomic neuroendocrine stimulation of lipolysis, and increased catecholamine release, which are partially responsible for stimulating higher lipid usage (Workman and Bassett 2012). Reduced glucose concentrations following an acute exercise bout in hypoxia have also been observed when compared to a normoxic exercise bout (Bailey et al., 2015). This supports the preference for glucose derived fuels in hypoxia during both rest and exercise. Greater contribution of fat oxidation following acclimatisation (Young et al. 1982), is likely to be due to an energy imbalance (Braun, 2008; Marriott and Carlson, 1996) not dissimilar to the observed effects of starvation (Saudek and Felig 1976).

A dampening of the previously observed increase in blood glucose dependency at altitude can occur through restriction of calories (Young et al., 1982). Similarly a reduced calorie intake may also result in a disruption in the acclimatisation process highlighted through attenuated insulin and EPO concentrations compared to adequately fed individuals (Barnholt et al., 2006). Furthermore, a blunted response of adrenaline availability at altitude due to the negative effect of calorie restriction may acutely decrease CHO availability and usage, due to a direct action on muscle glycogenolysis and an indirect action on hepatic glucose production (Barnholt et al., 2006). A marked reduction in fatty acid consumption following rest and during exercise has been observed acutely and following acclimatisation at altitude in men fed sufficiently to cover need. This again highlights glucose to be the predominant fuel at altitude in those who are not in a negative energy balance (Roberts et al. 1996).

In the present study, upon return to SL an increase in the utilisation of fat in comparison to ALT12 and ALT18 was recorded. Although no significant alteration was recorded at POST46 compared to PRE values, a 9% increase in contribution of EE from FAT was recorded. Increased contribution of fat following the sojourn compared to measures at altitude may also in the present study be attributable to the reduction of body mass and a subsequent increase of fat contribution.

BLOOD MEASURE RESPONSES

No changes in FFA or TAG were observed following the 18 day stay at 3,400m. Free fatty acids and glucose concentrations appear unaffected by altitude exposure. Although present data does not convey information regarding the delivery or use of such substrates previous work has demonstrated no reduction in the delivery of FFA or glucose upon exposure to altitude (Brooks et al. 1991; Brooks 2014). As such arterial glucose levels are unaffected during acute altitude exposure and decrease by 5-7% after longer term exposure to 4,300m (Brooks et al., 1991; Roberts et al., 1996). Increases in circulating FFA, during chronic stay at altitude have been interpreted as an increase in lipid utilisation at altitude, an effect previously seen in both rats (Yin et al., 2009) and humans (Jones et al., 1972). Increases in circulating FFA however do not necessarily translate to an increased rate of lipid oxidation (Roberts et al., 1996). A decrease in the uptake of FFA and glycerol by resting muscle has been reported in conjunction with increased plasma levels of FFA and glycerol following exposure to 4,300m and an increased dependence on blood glucose for metabolic fuel (Roberts et al., 1996).

Rather than directly promoting an increase in thermogenic gene programme; *Metrnl* stimulates several immune cell subtypes to activate the pro-thermogenic actions of adipose tissue after entering it. This occurs by an action upon the adipocytes and the process is increased upon acute but not chronic exposure to cold conditions in mice compared to neutral temperature controls (Rao et al., 2014). The measures taken in this study indicate that an 18 day residence at a moderate altitude of 3,400m in neutral temperatures (24.2°C and 33.9% RH) does not illicit changes in plasma *Metrnl* when measured within one week of return to sea-level. Previous work from Rao et al. (2012) reported no change in *Metrnl* expression following chronic exposure (two weeks) to cold conditions (4°C).

Currently the temporal changes that occur following stimulation of *Metrnl* regarding both its signalling and expression are unknown. As such it is possible that a transient increase and a subsequent decrease to pre-stimulation levels may have occurred in the present study during the 18 day stay at altitude. The measurement of *Metrnl* concentration prior to exposure and upon return to SL and not during altitude exposure may therefore be a reason for the observed lack of change in concentration. A lack of change in %BF in the present investigation may suggest that if any transient increase in *Metrnl* did occur, it was insufficient to induce changes in body composition however the length of residence and the severity of altitude much be considered and may also be a contributing factor to lack of observed change in the present study. Nevertheless previous work has recorded altered body composition following 21 days at an altitude ranging from 900 to 5,100m (Macdonald et al., 2009). Moreover in

transfected mice, a 25% reduction in whole body-fat content was reported following a 20-fold increase in liver *Metrn1* mRNA and a 5-6 fold increase in plasma *Metrn1* following three days. Further, increased EE in mice, five days post-injection of *Metrn1*-expressing adenoviral vectors, was displayed with improved glucose tolerance and no change in RER, food intake or physical activity. The time course of the reported increase is consistent with the time course of thermogenic gene expression suggesting that *Metrn1* may not directly regulate thermogenesis rather regulate biological processes that promote increased browning of white fat (Rao et al., 2014). Future work should focus on differing severities and length of exposure to altitude as to further illuminate effects of environmental conditions on *Metrn1*. This study fits within the thesis by investigating a period of altitude living and the metabolic effects associated with this environment following the examination of acute hypoxia on similar measures and prior to the studying of lasting metabolic effects in study 5 (Chapter 8).

LIMITATIONS AND FUTURE DIRECTIONS

The present study used recreationally active, normal weighted participants and thus findings of the study are limited to this population. The use of healthy participants allows for the investigation of the physiological effects of altitude without the confounding factor of obesity especially when looking at a novel aspect such as *Metrn1*. Future work should focus on the effect of altitude of differing severities and durations on *Metrn1* and taste sensation. The release of adipose tissue hormones such as Leptin and Ghrelin may mediate reductions in feeding (Smith et al., 2011) which must be considered in the present investigation as a possible altitude induced mechanism. A lack of physical activity and dietary data is a limitation and cannot be excluded as possible causes for loss of body mass during the sojourn. These data are consistent with the cachexia inducing effects of altitude stay and provide novelty through the effect of altitude on *Metrn1* however a lack of measurement of *Metrn1* at altitude is a limitation to the study. Nevertheless we added to information from previous studies by measuring *Metrn1* and markers of appetite in conjunction with measures relating to sensations of taste. The maintenance of body mass during a follow up period is in agreement with previous work. Future investigations should further investigate the mechanistic effect of altitude exposure of differing magnitudes and time periods on fat "browning". Moreover the lasting effects of an intermediate length stay at altitude should also be focused upon as such, the following experimental chapter (study 5/ Chapter 8) will investigate any lasting effects on blood lipid response at both one and four weeks upon return. Dietary composition can have an effect on RER and substrate use with a high fat diet previously shown to reduce measures of RER in fed mice (Marvyn et al., 2016). In order to control for the effect of diet on substrate use, RER and metabolic rate in the present study measures were taken at three points throughout the altitude stay during which participants had been fasted for the previous 12 hours. Nevertheless substrate use, RER and RMR data must be viewed in light of these dietary effects.

7.6 CONCLUSION

An 18 day stay at 3,400m induced significant and lasting reductions in body mass, and an increase in CHO contribution to metabolic processes. Post-altitude fat oxidation was unchanged upon return to sea-level compared to pre-altitude values. There were reductions in sensation of taste, suggesting this may influence food intake or choice and provide a mechanism for changes in eating habits at altitude. Correlation between greater changes in body mass and increased RMR and between greater changes in body mass and reduced SpO_2 following the 18 day stay indicate that individual response to altitude may influence the magnitude of weight loss through increases in metabolic rate. Greater hypoxemia, measured through SpO_2 , is seemingly linked to greater losses of body mass in a healthy population at moderate altitude.

8. THE EFFECTS OF A MODERATE ALTITUDE STAY ON POSTPRANDIAL METABOLIC PARAMETERS

8.1 ABSTRACT

AIM: Prolonged stay at altitude has been shown to induce lasting effects upon return to sea-level on metabolic parameters including an increase in basal metabolic rate (Lippl et al. 2010), reductions in postprandial insulin, and a reduction in body mass and fasting serum total cholesterol levels (Debevec et al., 2014). The present study assessed the effects of an 18 day stay at 3,400m on fasting and postprandial blood lipid and insulin levels before travel and one and four weeks after return to sea-level. The study also examined postprandial substrate oxidation before travel and one and four weeks post-return to sea-level following an 18 day stay at 3,400m. It was hypothesised that an intermediate length altitude stay would alter the postprandial lipid and insulin response upon return to sea-level. Specifically an increase in fat use is hypothesised based upon previous work from Workman and Bassett (2012). **METHOD:** Ten individuals (5 males, 5 females; age, 23 ± 4 yr.; BMI 24 ± 2 kg/m²) visited the laboratory on three occasions. One prior to an 18 day stay at 3,400m in Cusco, Peru (PRE) and two visits upon return to sea-level within one (POST1) and four weeks (POST2) respectively. Measures of blood lipids (FFA, TAG, CHOL, LDL, and LDL), insulin, resting metabolic rate (RMR) and substrate utilisation prior to and following the ingestion of a high lipid meal were recorded at each 60 minute time point over a period of 240 minutes. **RESULTS:** Following an 18 day stay at 3,400m lasting reductions in body mass were observed. Contribution to EE from carbohydrate (CHO) derived fuels increased at altitude compared to sea-level (study 4/ Chapter 7). A high lipid drink prior to the altitude stay increased circulating plasma FFAs ($+0.155 \pm 0.031$ mmol.L⁻¹, $p = 0.02$), TAG ($+0.65 \pm 0.35$ mmol.L⁻¹, $p = 0.03$), total CHOL ($+0.78 \pm 0.47$ mmol.L⁻¹, $p = 0.002$) insulin ($+58.94 \pm 9.54$ μ IU ml⁻¹, $p < 0.01$) and glucose ($+1.85 \pm 0.45$ mmol.L⁻¹, $p < 0.01$). An 18 day stay at 3,400m had no lasting effect on postprandial measures at one and four weeks return to sea-level. Measures of blood lipids, insulin, substrate oxidation and RMR were similar across trials ($p > 0.05$). **CONCLUSIONS:** An intermediate length high altitude exposure to 3,400m does not alter postprandial responses to a high fat meal upon return to sea-level in both the short (one week) and long (four weeks) term. This suggests that there is no lasting, metabolic effect of this type of altitude exposure following a high fat meal and the mechanism for reduced weight observed in the literature does not seem to be due to altered digestion, absorption and metabolism of lipids. Further research is required to determine whether there are other protective metabolic consequences of altitude exposure upon return to sea-level.

8.2 INTRODUCTION

Exposure to environmental hypoxia has been highlighted as a potential method to induce beneficial effects for obesity and the metabolic syndrome. Populations residing at altitude and lowlanders exposed both acutely, in the long term and coupled with or independent of physical activity, demonstrate improvements in various markers of health, as investigated in previous chapters of the current thesis. The extent to which metabolic and physiological responses at altitude occur in humans are due, in part, to the level of elevation above sea level, the resulting severity of hypoxia and the length of stay at a given altitude (Lenfant and Sullivan, 1971). The extent to which hypoxia induced physiological changes remain upon return to sea level in the non-acclimatised lowlander following an intermediate term exposure to moderate altitude, however, is not well established. The present study sits within the overall aim of the thesis through investigating the longer term sustained effects of a period of altitude living on common measures taken throughout the thesis. The study of lasting effects of an altitude stay comes after the investigation of the effects of acute hypoxia, acute hypoxia coupled with exercise and the immediate effects of a period of altitude living.

Upon exposure to high altitude mean losses of body mass equating to approximately 150 – 200 g/day are reported with a contributory energy shortfall of 500kcal.day⁻¹ suggested as common. Contributing mechanisms include an increase in basal needs, reduced energy intake (Westerterp 2001; Mawson et al. 2000; Lippl et al. 2010; Kayser and Verges 2013; Westerterp-plantenga et al. 2011; Westerterp and Kayser 2006) and alterations in substrate use (Braun et al. 2000; Roberts, 1996; Brooks 2014).

Chronic stay at altitude has been associated with a number of beneficial cardio-metabolic adaptations in humans. An inverse dose-response relationship between the elevation at which an individual resides and the prevalence of obesity has recently been demonstrated in a cross-sectional study representative of 207 million Americans (Voss et al., 2013). People living at high altitude (3,250-4,500m) have lower reported blood glucose levels (Picon Reategui 1963; Castillo et al., 2007) and lower risk of type 2 diabetes (Zubiarte 2001) than individuals resided at sea-level. Lower homeostatic model assessment (HOMA) values are also observed in humans living at high altitude (4,100m) suggesting greater insulin sensitivity in these populations (Baracco et al. 2006 Lindgarde et al 2004). Moreover coronary heart disease mortality rates are lower in populations living at an altitude above 1,220m (Mortimer et al., 1977) and serum high density lipoprotein cholesterol levels are higher in individuals residing between 3,000 and 5,500m than those at lower altitude (Sharma 1990 in Goto 2015) suggesting some cardio-metabolic beneficial effects exist from residing at altitude. Taken together these findings provide evidence for a potential protective effect of chronic altitude stay in some parameters of health and of a role of altitude residence in the long term homeostasis of body mass.

Beneficial effects of acute environmental hypoxia have also been demonstrated in lowlanders. Work from Kelly et al., (2010) demonstrated a preventative effect of an acute severe hypoxic exposure

(4,300m) on postprandial hyperglycemia, illustrated by attenuated peak glucose values following a 75g glucose load in simulated normobaric hypoxia versus a sea-level control glucose tolerance test. More recently Goto et al (2015) concluded that acute exposure to a moderate hypobaric hypoxia equivalent to 2,500m may be beneficial in augmenting carbohydrate oxidation without affecting the glucose or insulin response. This was demonstrated in healthy males, where an increased RER was observed following a 75 g glucose load in a hypobaric chamber compared to values in normoxic conditions. This illustrates that following the same glucose load an increase in RER observed in hypoxia compared to normoxia supports the role of hypoxia per se on augmenting carbohydrate oxidation. Work from Mackenzie et al., (2011) reported an improved fasting insulin sensitivity in type 2 diabetic patients following 60 minutes rest in simulated hypoxia (FiO₂:14.6%) during which a mean SpO₂ of 92% was observed. Postprandial responses in hypoxia, following a breakfast meal consisting of a third of daily caloric intake and 32.5g of fat per m² of body surface, have been shown to induce a net stimulation of lipolysis of plasma TAG when compared to postprandial responses in normoxia (Ferezou et al., 1993). This was highlighted through a blunting of CHOL, TAG and phospholipid concentrations four hours following ingestion of a meal, suggesting a protective effect of hypoxia as far as lipaemia is concerned. These results however must be viewed with caution as the nature of the point measurement of blood does not inform of the dynamic process involving absorption, digestion and removal (storage and metabolism).

On the contrary other studies have reported increased insulin resistance and hyperglycaemia following acute exposure to environmental hypoxia (hypoxia was induced by decreasing oxygen saturation to 75% for 30 minutes) (Oltmanns et al., 2004) and intermittently (85 – 95% SpO₂ for eight hours) (Louis and Punjabi 2009). Elevated release of adrenaline (+ 25%) upon exposure to hypoxia is one suggested mechanism for acute glucose intolerance upon exposure (Oltmanns et al., 2004). Although the exact underlying mechanisms for the acute intolerance are unclear, it is suggested that an influential mechanism is likely an increased hepatic glucose output and decreased muscular glucose uptake (Baron et al. 1987;Oltmanns et al. 2004).

Prolonged stay at altitude has also been shown to induce beneficial alterations in markers of cardiovascular disease risk not only in high altitude dwellers but in lowlanders who travel to altitude. Published research shows: decreased body mass, reductions in levels of high-density lipoprotein and diastolic blood pressure following a seven day stay at 2,650m in an obese group of participants (Lippl et al., 2010). Likewise reduced peak capillary blood glucose values following an oral glucose tolerance test (OGTT) and improved HOMA index, as a measure of insulin resistance, were recorded in individuals with metabolic syndrome, in conjunction with short term favourable effects in heart rate and systolic blood pressure monitored over a 24 hour period during a 21 day stay at 1700m (Schoesberger et al., 2003). Importantly lasting effects of short term residence at altitude upon return to sea-level are observed in these same measures. Reductions in systolic blood pressure remained up to seven to 10 days upon return to sea-level (500m) from altitude (Schoesberger et al., 2003).

Increases in metabolic rate, reductions in HDL and increases in LDL have been recorded one week upon return to sea level whilst reduced energy intake, reduced body mass, reduced diastolic blood pressure and improved walking distance during a 6 minute walk test have all been observed in obese individuals five weeks post-return to sea-level following seven days at 2,650m (Lippl et al., 2010) suggesting lasting improvements may exist from hypoxia induced alterations. Similarly three days of moderate altitude living (2,400m) for untrained individuals was sufficient to improve glucose tolerance, measured through an oral glucose tolerance test (OGTT) (Lee et al., 2003). Lasting reductions in body mass for up to four weeks following an intermediate length stay at 3,400m are reported in study 4 (Chapter 7) of this thesis.

Exercise training coupled with exposure to environmental hypoxia also represents a relatively new approach to improve symptoms of metabolic syndrome. Decreased body mass (Netzer et al., 2008), body fat mass (Wiesner et al., 2010), blood pressure (Schobersberger et al., 2003) and arterial stiffness (Vedam et al., 2009) were observed in individuals completing moderate intensity exercise in conditions of hypoxia. Exercise training coupled with hypoxia also induces improvements in insulin sensitivity compared with equivalent training in normoxia, in both healthy individuals (Haufe et al., 2008) and those with type 2 diabetes (Mackenzie et al., 2011). Postprandial insulin sensitivity has also been shown to be significantly improved following a four week exercise programme in moderate hypoxia (FiO₂: 0.15) when compared to a normoxic training programme (Morishima et al., 2013). These findings suggest that a hypoxic stimulus facilitates glucose metabolism during exercise, arising in part from improved insulin sensitivity. An acute exposure to a severe normobaric hypoxia prior to a moderate intensity cycling exercise bout in normoxia was examined in study 3 (Chapter 6) of the current thesis. A passive exposure to hypoxia was seen to elicit increased EE through greater fat and CHO use with predominance from CHO sources. Interestingly moderate intensity exercise was seen to be useful for lowering postprandial hypertriglyceridemia following a high fat meal, although a prior hypoxic exposure did not induce acute alterations in substrate oxidation during a subsequent exercise bout. This suggests that significantly greater fat oxidation does not occur during moderate intensity exercise, as compared with rest where it has been shown to increase during post-hypoxic exposure (Workman and Basset 2012).

Despite these improvements in health related functions, exposure to high and extreme altitude (> 4,000m) may lead to negative effects in un-acclimatised individuals, including symptoms of acute mountain sickness. The use of severe hypoxia may not, therefore, be appropriate for the treatment of people with significant disease or health complications, especially those with cardiovascular and respiratory disease or disorders. The effect of an intermediate length stay (>10 days) at moderate altitude on postprandial metabolism upon return to sea-level is unknown. It is predicted that an 18 day stay at 3,400m will alter postprandial metabolism thus accounting for changes in body mass. Greater information on this topic may be helpful in improving the understanding of the lasting effects of moderate altitude on substrate oxidation and the postprandial insulin response. This, in turn, may

provide useful information for the utilisation of exposure to an environment of hypoxia as a therapeutic tool in unhealthy individuals such as those with cardiovascular and/ or metabolic conditions. Therefore the primary aims of this study were to 1. Assess the effects of an 18 day stay at 3,400m with no structured exercise intervention on the postprandial insulin response before and within one week of return to sea-level. 2. Assess the effects of an 18 day stay at moderate altitude (3,400m) with no structured exercise intervention on the postprandial insulin response within four weeks upon return to sea-level. 3. Examine postprandial substrate oxidation upon one and four weeks post-return to sea-level following an 18 day stay at 3,400m. It was hypothesised that an 18 day stay at moderate altitude will alter the postprandial lipid and insulin response to a high fat load within one week upon return to sea level but that these will cease four weeks upon return. As seen upon acute and short term exposure an increase in fat use is hypothesised upon return to sea-level (Workman and Basset, 2012).

8.3 MATERIALS AND METHODS

PARTICIPANTS

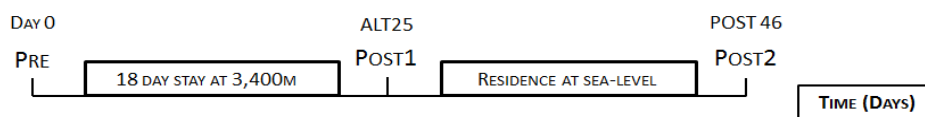
Ten individuals (5 males, 5 females) [23 ± 4 years of age (mean \pm SD), body mass 73 ± 11 kg, height 175 ± 10 cm and BMI 24 ± 2 kg/m²] agreed to undertake the study and complied with all criteria for participation [(3.5.1) Medical criteria and recruitment and (3.6) Pre-trial diet and exercise standardisation and (3.8) Hydration assessment]. Prior to the undertaking of the experimental trials volunteers attended the laboratories whereby their anthropometric data was collected [(3.11) Anthropometric assessment]. Female participants were tested throughout the menstrual cycle thus the use of contraceptive medication is likely.

EXPERIMENTAL DESIGN

Participants visited the laboratory on three occasions. All trials were identical in their design and conducted at sea-level. The experimental design consisted of a trial within one week prior to an 18-day stay at moderate altitude (3,400m) in Cusco, Peru (PRE), a trial upon return to sea-level (SL) at POST25 (POST1) and a trial at POST46 (POST2) these were seven and 28 days post-return to sea-level. Each trial lasted for 240 minutes.

Following the PRE trial participants travelled by air from London to Cusco (3,400m). Ambient temperature and relative humidity during the altitude stay were measured on three occasions at 24.1°C and 36.5% at ALT5, 26.3°C and 30.2% at ALT12 and 22.2°C and 35% at ALT18. During the altitude stay participants were instructed to eat and drink *ad libitum* so as to reflect the effects of an altitude sojourn rather than to impose the effects of a strict diet. The altitude sojourn included activities comparable to the lives of the participants at sea-level. No structured exercise programme was administered to the participants during the altitude stay. The experimental design is illustrated in Figure 8.1.

EXPERIMENTAL DESIGN



TRIAL DESIGN

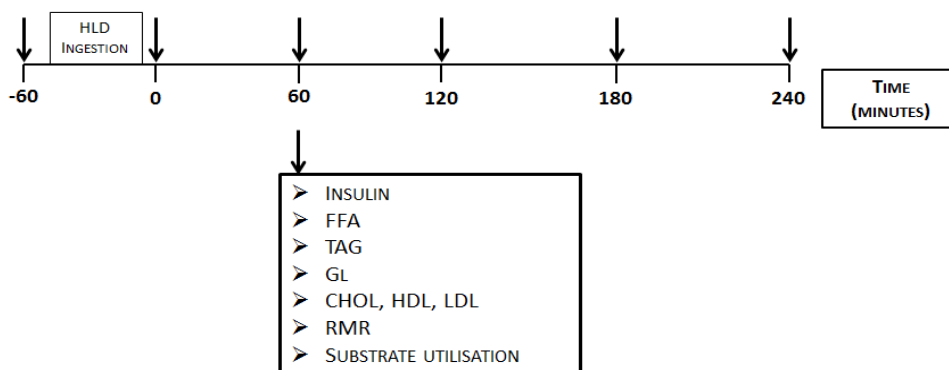


Figure 8.1: Schematic of study design. FFA, free fatty acids; TAG, triglycerides; GL, Glucose, CHOL, Total Cholesterol, HDL, High density lipoprotein, LDL, Low density lipoprotein, RMR, Resting metabolic rate.

GAS MEASURES AND PHYSIOLOGICAL DATA

Expired gas was collected in Douglas bags over a recorded period of time lasting approximately 60 seconds every 30 min during each experimental period, as described in chapter 3.13.1. Resting EE and substrate partitioning was estimated indirectly via non-protein respiratory exchange ratio (RER), which was determined through expired gas, assuming constant protein oxidation. Oxidation rates of fat and carbohydrate were calculated according to the equations of Peronnet and Massicotte (1991) [(3.13.3) Calculations derived from cardiopulmonary measures]. Prior to the measurement of resting metabolic rate individuals were required to rest for 30 min in a supine position in a quiet environment. The participants were instructed to remain quiet in a supine position during data collection but to remain awake.

BLOOD MEASURES OF LIPID METABOLISM

Two baseline venous blood samples were taken [(3.17.1) Cannulation] on arrival to the laboratories (PRE1 and PRE2) separated by 30 minutes. Subsequently samples were taken at min 0 and from then on at each 60 min time point. With plasma separated [(3.17.2) Blood plasma separation] FFA [(3.17.3) and TAG (3.17.4) were analysed. Insulin was analysed using a quantitative sandwich enzyme immunoassay technique (Sigma Aldrich, UK) with an intra-assay and inter-assay precision of CV% < 3.9% as per manufacturer instruction.

Analysis of high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (CHOL) and glucose (Gl) were analysed following a fingertip capillary blood sample (Accucheck Softclix Pro, Roche,

Lewes, England) using the CardioChek PA Meter kit (CardioChek, USA). Homeostasis model assessment (HOMA) as a marker of insulin resistance was calculated using the equation proposed by Matthews et al. (1985). The HOMA index is derived from the balance between hepatic glucose output and insulin secretion from fasting levels of glucose and insulin. The HOMA index requires measurement of insulin and glucose in the basal state.

HOMA equation proposed by Matthews et al. (1985);

$$\text{Fasting Insulin} \times \text{Fasting Glucose} \div 22.5$$

$$I_0 \times G_0 / 22.5$$

TEST MEAL

Participants were provided with a high-lipid meal in a liquid form and instructed to consume the drink in less than 10 minutes. Participants were provided with the high-lipid meal at minute 0 following baseline measures. The meal consisted of a combination of 180 mL double cream and 110 g of ice cream. Similar meals have been successfully used in previous studies to induce postprandial hypertriglyceridemia (PHTG) (Zhang et al., 2007). The HLD provided 883 kcal, 73 g fat, 45 g CHO, (32 g sugar) and 9 g protein.

STATISTICAL ANALYSIS

All data were checked for normality and sphericity [(3.15.2.1) Normal distribution and (3.15.2.2) Sphericity] and were adjusted using the Huynh-Feldt method. All data were analysed using a standard statistical package (SPSS version 20 for Windows 7).

A two-way analysis of variance (ANOVA) with repeated measures for trial (PRE, POST1 and POST2) and time (6) was conducted for all measures of insulin, FFA, substrate oxidation and measures of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and RER. Bonferoni-pairwise comparisons were used to compare between separate time points throughout the testing time frame. Effect sizes reported are Cohen's *d* and partial eta squared (η^2). The recommendations for these calculations are based upon the review of Lakens (2013). Effect sizes for main effects and interactions are presented as partial eta squared (η^2), while differences between two related samples were evaluated through Cohen's d_{av} in accordance with Lakens (2013).

The area under the curve (AUC) values was calculated using the conventional trapezoid method. A general linear model, one-way, repeated measures analysis of variance was used to identify main effect of condition. Main effects were followed up using Bonferoni pairwise comparisons comparing separate conditions.

8.4 RESULTS

BLOOD LIPID RESPONSES

A difference in FFA values was observed over time ($F_{(5, 25)} = 14.195$, $p < 0.001$, $np^2 = 0.740$). Increases in FFA were observed at three hours ($p = 0.02$, $d = 7.28$) and four hours ($p = 0.02$, $d = 8.69$) post-high lipid meal compared to one hour post-ingestion. An increase at three hours post-ingestion was also recorded compared to PRE value ($p = 0.05$, $d = 5.47$). Mean differences of $0.135 \pm 0.130 \text{ mmol.L}^{-1}$ were seen at three hours post-ingestion compared to PRE values whereas mean increases of $0.154 \pm 0.114 \text{ mmol.L}^{-1}$ and $0.104 \pm 0.042 \text{ mmol.L}^{-1}$ were observed at three and four hours post-ingestion respectively compared to one hour post-ingestion values.

No difference in FFA AUC values were observed across trials ($p > 0.05$). AUC values of 1.65 ± 0.37 , 1.61 ± 0.18 and $1.56 \pm 0.14 \text{ mmol.L}^{-1} \cdot \text{hr}$ were recorded in Pre, POST1 and POST2 trials respectively. Free fatty acids values across the three trials are displayed in Figure 8.2.

A difference in TAG values was observed over time ($F_{(5, 25)} = 14.524$, $p < 0.001$, $np^2 = 0.890$). Increases in TAG were observed at three hours ($p = 0.01$, $d = 11.9$) and four hours ($p = 0.03$, $d = 10.3$) post-high lipid meal compared to PRE ingestion values. Mean values of $1.41 \pm 0.19 \text{ mmol.L}^{-1}$ and $1.41 \pm 0.24 \text{ mmol.L}^{-1}$ were recorded at three and four hours post-ingestion respectively, compared to a mean value of $0.77 \pm 0.19 \text{ mmol.L}^{-1}$ prior to the high lipid meal. Triglyceride values across the three trials are displayed in Figure 8.2.

Similarly a difference in TAG AUC values was observed over time ($F_{(4, 20)} = 50.811$, $p < 0.001$, $np^2 = 0.910$) with increases seen from pre-meal values to one, two and three hours post-ingestion ($p < 0.05$).

An interaction effect was observed between trial and time ($F_{(8, 40)} = 2.257$, $p = 0.043$, $np^2 = 0.311$).

AUC values at two to three hours post-ingestion were higher in the POST2 trial (%) compared to Pre.

AUC values of 4.85 ± 0.47 , 5.40 ± 0.46 and $5.53 \pm 0.50 \text{ mmol.L}^{-1} \cdot \text{hr}$ were recorded in Pre, POST1 and POST2 trials respectively.

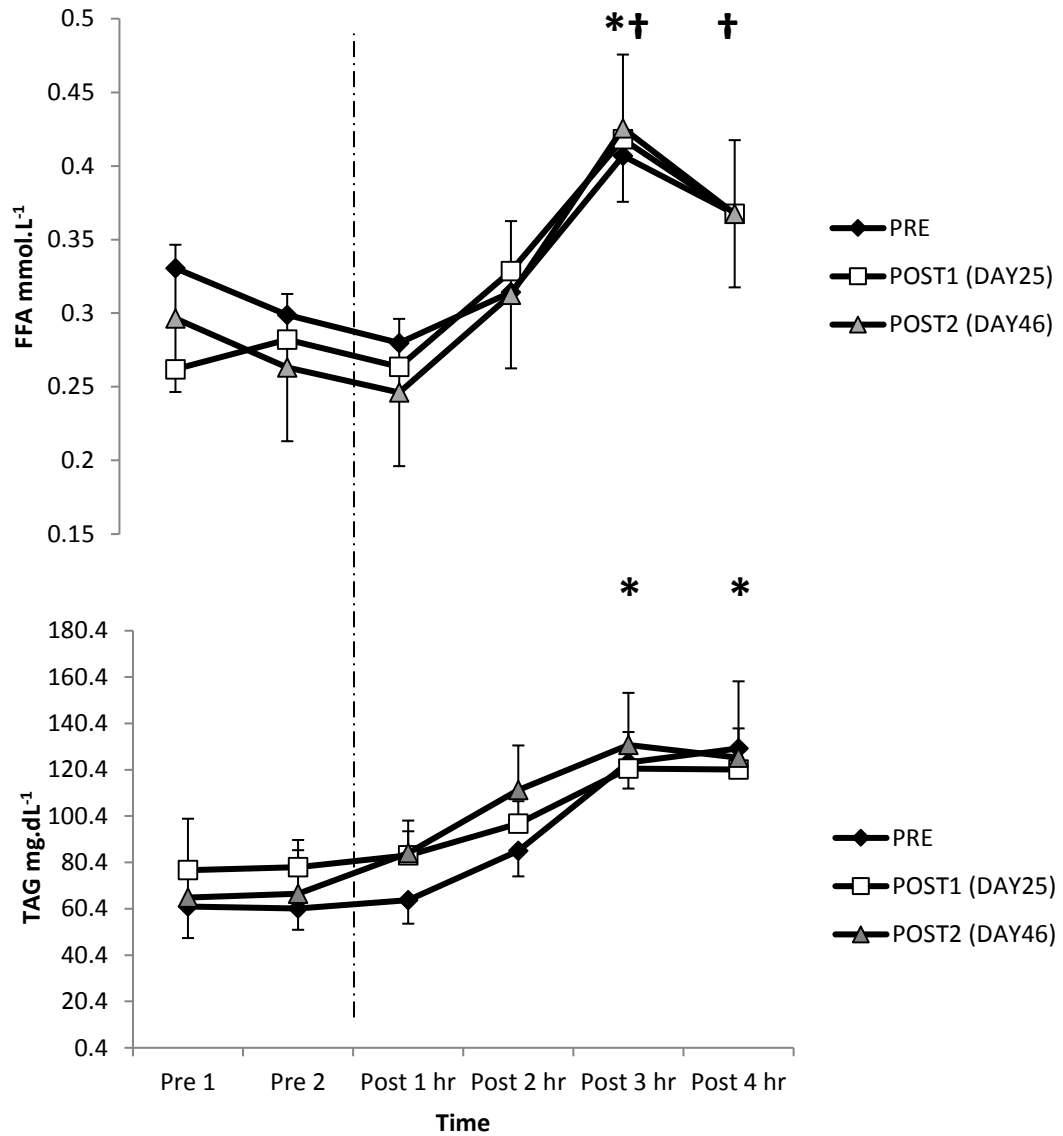


Figure 8.2: Plasma FFA and TAG concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2). * indicates significance compared to Pre1 values. † indicates significance compared to Post 1 hr values. All significance symbols correspond to $p < 0.05$. Dashed line indicates the time at which the meal was ingested.

GLUCOSE AND INSULIN RESPONSE

A difference in glucose values was observed over time however ($F_{(5, 25)} = 24.756$, $p < 0.001$, $np^2 = 0.900$). Increases in glucose were observed at 2 ($p = 0.003$, $d = 11.14$) (5.55 ± 0.58), 3 ($p < 0.001$, $d = 18.95$) (5.94 ± 0.39) and four hours ($p = 0.009$, $d = 12.1$) (5.39 ± 0.68) post-high lipid meal compared to PRE ingestion values (4.09 ± 0.16). All values are shown in Figure 8.4.

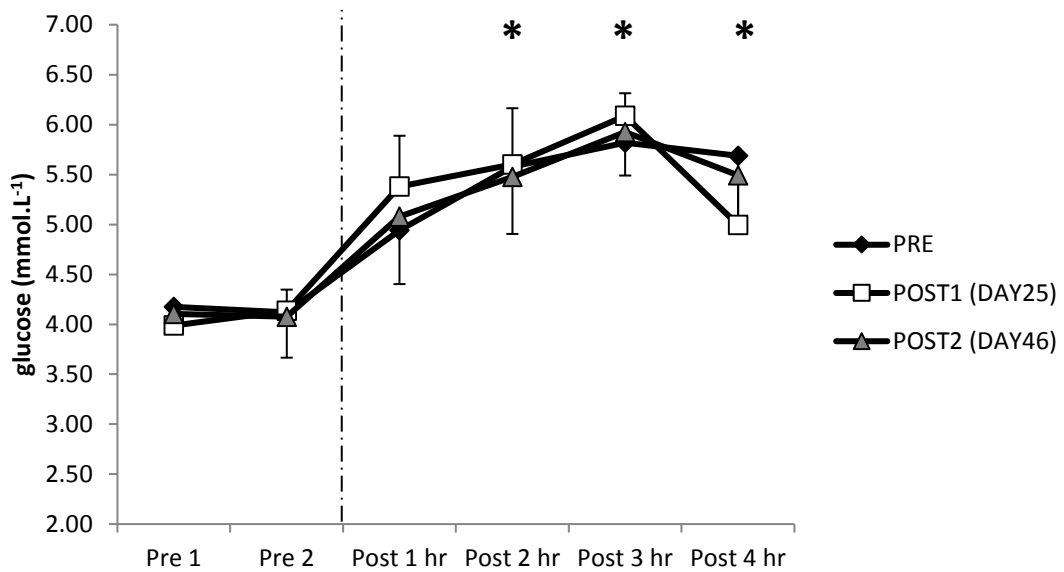


Figure 8.4: Plasma glucose concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2). * indicates significance compared to Pre1 values. All significance symbols correspond to $p < 0.05$. Dashed line indicates the time at which the meal was ingested.

A difference in insulin values was observed over time ($F_{(5, 25)} = 28.751$, $p < 0.001$, $\eta^2 = 0.989$). Increases in insulin were observed at 30 minutes ($p < 0.01$, $d = 8.78$) post-high lipid meal compared to PRE ingestion values. Greater values were also observed at one hour post-ingestion compared to PRE ingestion ($p < 0.01$, $d = 9.06$) and at two hours post-ingestion compared to PRE ($p < 0.01$, $d = 4.87$). There was a peak mean increase of $+ 58.94 \pm 9.54 \mu\text{IU ml}^{-1}$ at 30 minutes post-ingestion compared to PRE values. All values are shown in Figure 8.3.

No change in HOMA index values were observed over the three trials ($F_{(2, 10)} = 1.8$, $p = 0.202$, $\eta^2 = 0.274$). Values of 1.32 ± 0.26 , 1.38 ± 0.43 and 1.18 ± 0.38 were recorded respectively at rest during Pre, POST1 and POST2 trials.

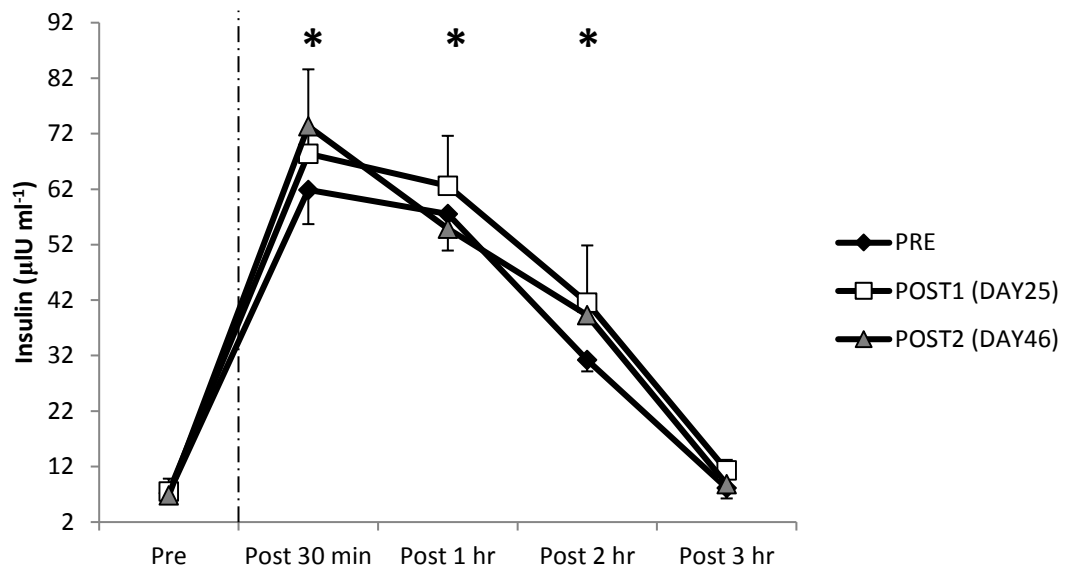


Figure 8.3: Insulin concentration ($\mu\text{U ml}^{-1}$) throughout the trial in all conditions (PRE, POST1 AND POST2). Measures were taken prior to ingestion of a high lipid drink (PRE), and at 30 minutes (Post-30min), 1 hour (Post-1hr), two hours (Post-2hr) and three hours (Post-3hr) post-ingestion. * indicates significant difference compared to Pre. All significance symbols correspond to $p < 0.05$. Dashed line indicates the time at which the meal was ingested.

OTHER BLOOD VALUE RESPONSES

A difference in CHOL values was observed over time ($F_{(5, 25)} = 18.751, p < 0.001, np^2 = 0.789$). Increases in CHOL were observed at four hours ($p = 0.002, d = 7.16$) post-high lipid meal compared to PRE ingestion values. Greater values were also observed at four hours post-ingestion compared to one hour post-ingestion ($p = 0.024, d = 5.87$). There was a mean increase of $0.78 \pm 0.47 \text{ mmol.L}^{-1}$ at four hour post-ingestion compared to PRE values. All values are shown in Figure 8.3.

No differences in fasting HDL between trials ($F_{(2, 10)} = 2.474, p = 0.134, np^2 = 0.331$) over time ($F_{(5, 25)} = 2.090, p = 0.100, np^2 = 0.295$) or as an interaction between trial and time was observed ($F_{(10, 50)} = 0.578, p = 0.824, np^2 = 0.104$). Similarly no differences in fasting LDL between trials ($F_{(2, 10)} = 0.059, p = 0.943, np^2 = 0.012$) over time ($F_{(5, 25)} = 0.444, p = 0.814, np^2 = 0.081$) or as an interaction between trial and time was observed ($F_{(10, 50)} = 1.322, p = 0.245, np^2 = 0.209$). Values are shown in Figure 8.5.

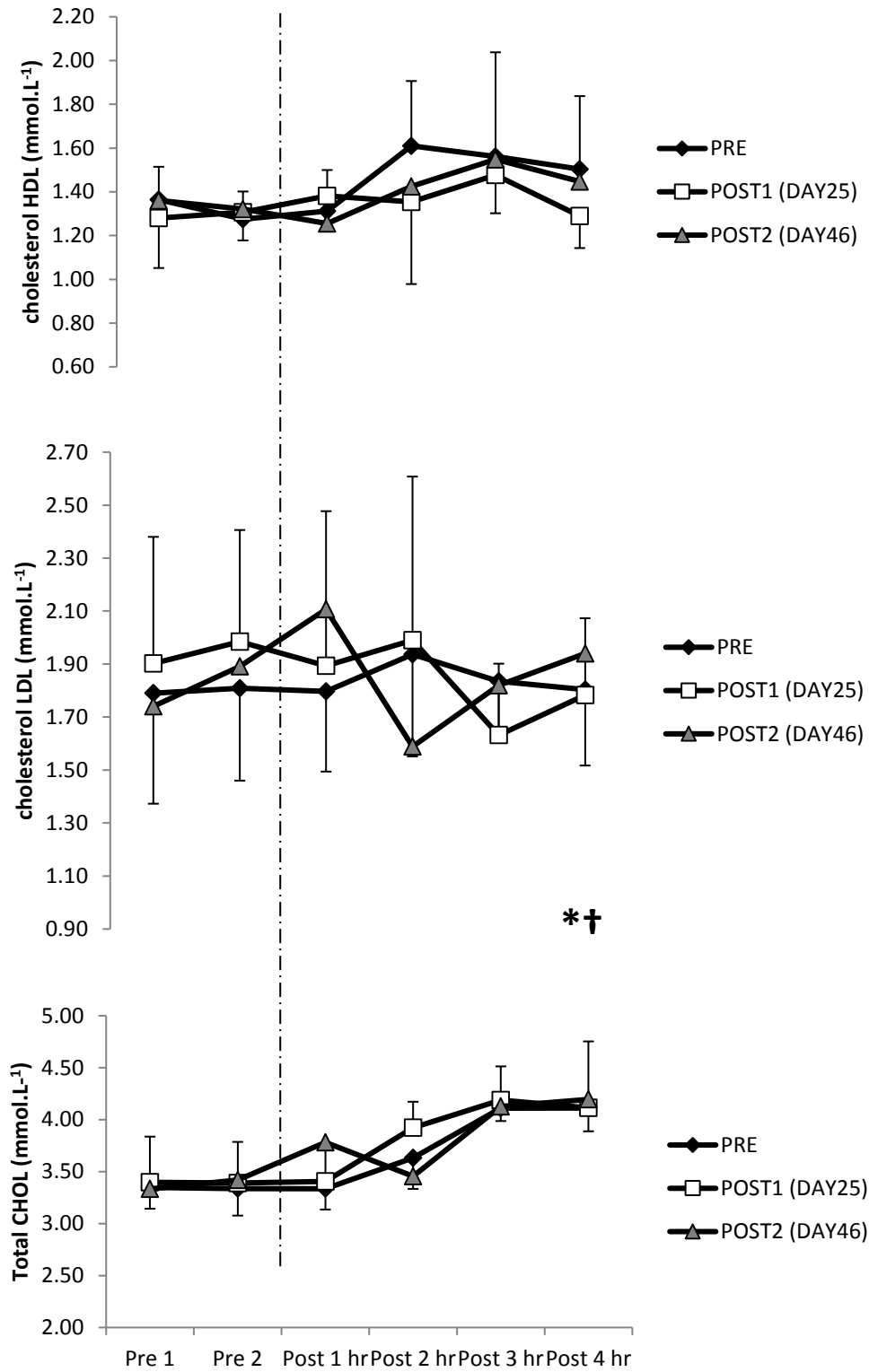


Figure 8.5: Plasma HDL, LDL and Total CHOL concentration (mmol.L⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2). * indicates significance compared to Pre1 values. † indicates significance compared to Post-1 hr values. All significance symbols correspond to p < 0.05. Dashed line indicates the time at which the meal was ingested.

RESTING METABOLIC RATE AND SUBSTRATE OXIDATION

A difference in RMR values was observed over time ($F_{(4, 32)} = 2.689, p = 0.049, np^2 = 0.252$). Peak RMR values of $1.56 \pm 0.25 \text{ kcal}\cdot\text{min}^{-1}$ and $1.53 \pm 0.13 \text{ kcal}\cdot\text{min}^{-1}$ respectively were observed at two hours following the high lipid meal during the post-altitude trials whereas peak RMR values for the PRE trials of $1.47 \pm 0.20 \text{ kcal}\cdot\text{min}^{-1}$ was observed at three hours after the high lipid meal. RMR values are displayed in Table 8.1.

There was no difference in values of CHO oxidation throughout the investigation between the three trials ($F_{(2, 16)} = 0.292, p = 0.750, np^2 = 0.035$). Peak CHO oxidation values of $0.21 \pm 0.08 \text{ g}\cdot\text{min}^{-1}$ and $0.20 \pm 0.07 \text{ g}\cdot\text{min}^{-1}$ were observed during PRE and POST1 trials at three hours post-ingestion whilst a peak value of $0.21 \pm 0.10 \text{ g}\cdot\text{min}^{-1}$ during POST2 trials was observed at two hours following the high lipid meal. All CHO values are displayed in Figure 8.6.

There was no difference in values of fat oxidation throughout the investigation between the three trials ($F_{(2, 16)} = 0.998, p = 0.390, np^2 = 0.111$). Peak fat oxidation values of $0.08 \pm 0.0 \text{ g}\cdot\text{min}^{-1}$, $0.10 \pm 0.02 \text{ g}\cdot\text{min}^{-1}$ and $0.09 \pm 0.03 \text{ g}\cdot\text{min}^{-1}$ were observed during PRE, POST1 and POST2 trials respectively at two hours post the high lipid meal. All fat oxidation values are displayed in Figure 8.6.

PHYSIOLOGICAL MEASURES

Accordingly there was no difference in RER between the three trials ($F_{(2, 16)} = 0.623, p = 0.549, np^2 = 0.072$). Mean RER values of 0.85 ± 0.05 , 0.84 ± 0.04 and 0.85 ± 0.05 were observed during PRE, POST1 and POST2 trials respectively. All RER values are displayed in Figure 8.6.

Values of $\dot{V}O_2$ were not altered between trials ($F_{(2, 16)} = 1.133, p = 0.347, np^2 = 0.124$). Similarly values of $\dot{V}CO_2$ showed no differences between trials ($F_{(2, 16)} = 0.720, p = 0.502, np^2 = 0.083$), over time ($F_{(4, 32)} = 2.549, p = 0.058, np^2 = 0.242$) or as an interaction between time and trial ($F_{(8, 64)} = 0.911, p = 0.513, np^2 = 0.102$). \dot{V}_E values remained similar between trials ($F_{(2, 16)} = 1.526, p = 0.087, np^2 = 0.113$). All physiological measures are displayed in Table 8.1

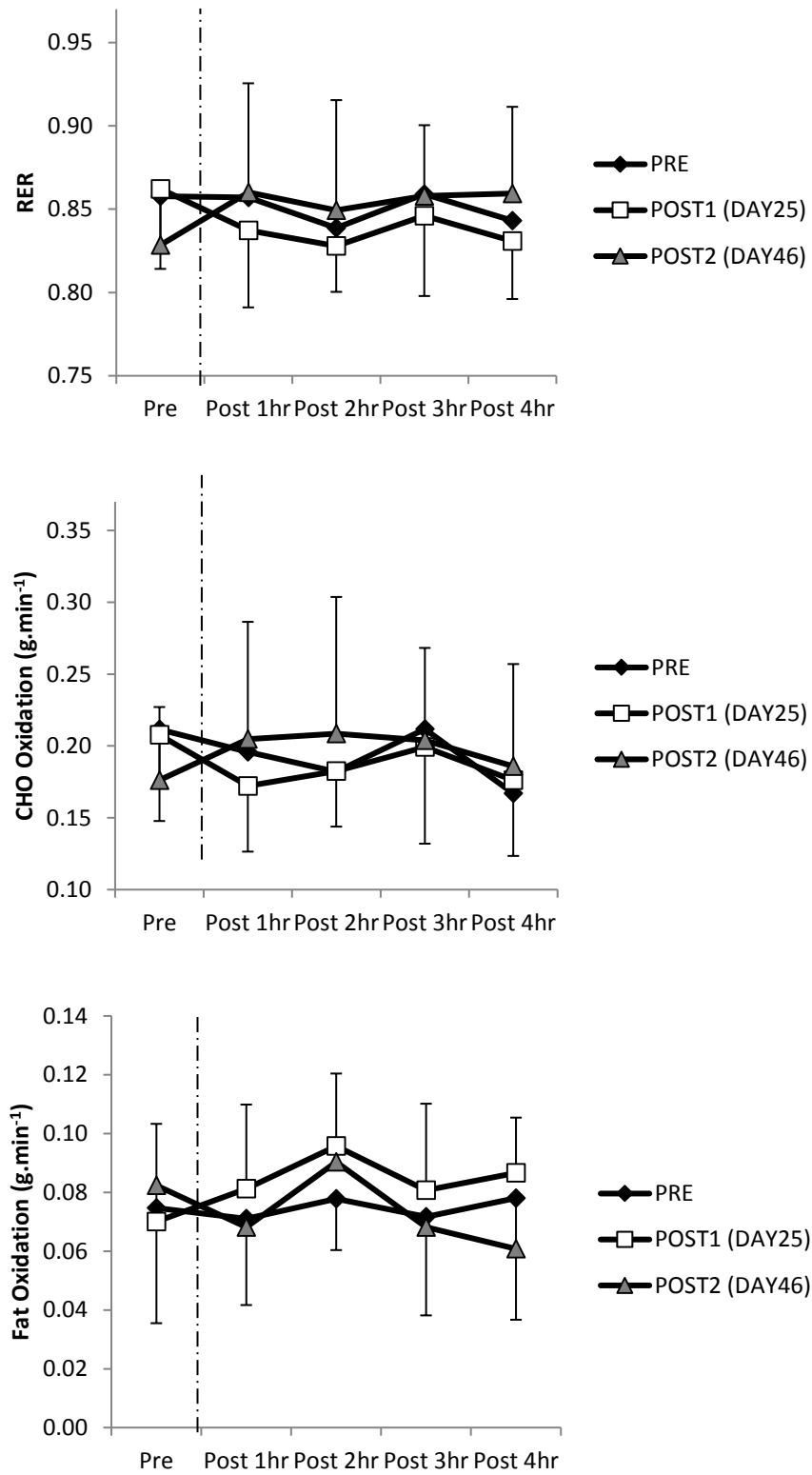


Figure 8.6: Respiratory exchange ratio, CHO oxidation (g.min⁻¹) and Fat oxidation values (g.min⁻¹) throughout the trial in all conditions (PRE, POST1 and POST2). Error bars for RER and CHO removed for clarity. Dashed line indicates the time at which the meal was ingested.

TABLE 8.1; Mean \pm SD data for physiological measures recorded at PRE, POST1 and POST2.

	PRE					POST1					POST2				
	Pre1	Post 1hr	Post 2hr	Post 3hr	Post 4hr	Pre1	Post 1hr	Post 2hr	Post 3hr	Post 4hr	Pre1	Post 1hr	Post 2hr	Post 3hr	Post 4hr
$\dot{V}O_2$ (L.min ⁻¹)	0.31 \pm 0.05	0.29 \pm 0.04	0.29 \pm 0.04	0.30 \pm 0.04	0.28 \pm 0.05	0.30 \pm 0.05	0.30 \pm 0.04	0.33 \pm 0.05	0.31 \pm 0.03	0.31 \pm 0.02	0.32 \pm 0.02	0.29 \pm 0.02	0.32 \pm 0.02	0.29 \pm 0.02	0.26 \pm 0.03
$\dot{V}CO_2$ (L.min ⁻¹)	0.27 \pm 0.04	0.25 \pm 0.04	0.25 \pm 0.04	0.26 \pm 0.04	0.24 \pm 0.03	0.26 \pm 0.04	0.25 \pm 0.03	0.27 \pm 0.04	0.27 \pm 0.03	0.26 \pm 0.02	0.26 \pm 0.02	0.25 \pm 0.02	0.27 \pm 0.03	0.25 \pm 0.03	0.23 \pm 0.03
\dot{V}_E (L.min ⁻¹)	5.7 \pm 0.6	5.7 \pm 1.1	5.9 \pm 1.7	6.4 \pm 1.1	4.7 \pm 0.7	5.6 \pm 1.3	6.2 \pm 1.8	6.7 \pm 2.2	7.1 \pm 2.0	7.2 \pm 1.7	0.9 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.05	0.9 \pm 0.05	0.8 \pm 0.03
RER	0.86 \pm 0.06	0.86 \pm 0.05	0.84 \pm 0.04	0.86 \pm 0.06	0.84 \pm 0.06	0.86 \pm 0.05	0.84 \pm 0.05	0.83 \pm 0.03	0.85 \pm 0.05	0.83 \pm 0.03	0.83 \pm 0.04	0.86 \pm 0.07	0.85 \pm 0.07	0.86 \pm 0.04	0.86 \pm 0.05
RMR (kcal.min ⁻¹)	1.50 \pm 0.23	1.36 \pm 0.22	1.41 \pm 0.22	1.47 \pm 0.2	1.36 \pm 0.21	1.44 \pm 0.23	1.40 \pm 0.19	1.56 \pm 0.25	1.52 \pm 0.15	1.48 \pm 0.09	1.52 \pm 0.11	1.41 \pm 0.10	1.53 \pm 0.13	1.42 \pm 0.13	1.28 \pm 0.15
CHO (g.min ⁻¹)	0.21 \pm 0.09	0.20 \pm 0.05	0.18 \pm 0.07	0.21 \pm 0.08	0.17 \pm 0.05	0.21 \pm 0.06	0.17 \pm 0.05	0.18 \pm 0.04	0.20 \pm 0.07	0.18 \pm 0.05	0.18 \pm 0.05	0.20 \pm 0.08	0.21 \pm 0.10	0.20 \pm 0.06	0.19 \pm 0.07
Fat (g.min ⁻¹)	0.07 \pm 0.04	0.07 \pm 0.03	0.08 \pm 0.02	0.07 \pm 0.03	0.08 \pm 0.04	0.07 \pm 0.03	0.08 \pm 0.03	0.10 \pm 0.02	0.08 \pm 0.03	0.09 \pm 0.02	0.08 \pm 0.03	0.07 \pm 0.03	0.09 \pm 0.03	0.07 \pm 0.02	0.06 \pm 0.02

8.5 DISCUSSION

The lasting effects of residence at high altitude on postprandial parameters upon return to sea-level are not well examined. Contrary to the hypothesis the present study observed no lasting effect of an 18 day stay at 3,400m on postprandial measures of blood lipids, insulin, RMR and substrate oxidation upon one and four weeks return to sea-level. In accordance with findings from study 3 (Chapter 6) of this thesis, a high lipid meal induced increases in plasma FFAs with peak increases observed three hours post-ingestion and values of TAG and glucose, yet evoked no alteration in postprandial substrate use. This study adds to the existing knowledge regarding the lasting effects of an altitude stay on metabolic parameters that may influence cardio-metabolic health. Specifically these results suggest that the intermediate effects of a stay at altitude may not be useful in inducing changes in substrate use or lipid profiles upon return to sea-level following a high fat load. It therefore seems unlikely that any post-altitude exposure effects on body mass (study 4/ Chapter 7) or composition arise from changes in the metabolic processing of foodstuffs. The present study sits within the overall aim of the thesis through investigating the longer-term sustained effects of a period of altitude living on common measures taken throughout the thesis. This study follows the investigation of acute hypoxia, acute hypoxia coupled with exercise and the immediate effects of a period of altitude living on metabolic parameters.

SUBSTRATE UTILISATION

Upon exposure to altitude losses of body mass are commonly observed often with sustained effects upon return to sea-level (Lippl et al. 2010; study 4/ Chapter 7). Body composition measurements indicate that losses of fat are predominant at moderate altitudes whereas a loss of lean tissue contributes to weight loss at altitudes of > 5,000m (Wing-Gaia, 2014). Causes for these changes include increased basal energy requirements, likely due to sympathetic activation, and the subsequent breakdown of energy stores (Butterfield et al., 1992; Marriott and Carlson, 1996) which have been measured upon four weeks of return to sea-level. Alterations in substrate use are commonly observed upon exposure and serve as a contributory mechanism is altered body mass. Increased utilisation of carbohydrate derived fuel sources of glucose, glycogen and lactate are observed upon acute exposure to hypoxia (Brooks et al. 1991; Morishima et al., 2014). Likewise following acclimatisation and chronic exposure to altitude increased CHO utilisation is also observed (Brooks et al., 1991; Roberts et al., 1996). Confounding variables including energy balance of the individual are present with regard to substrate use at altitude. An inhibition of the increased dependency on blood glucose is induced through calorie restriction (Young et al., 1982). In agreement with these previous findings; results from studies 2 and 3 (Chapters 5 and 6) in the present thesis demonstrate a greater CHO contribution following acute exposure to severe normobaric hypoxia. Greater CHO contribution was also measured at rest during an 18 day stay at 3,400m (study 4/ Chapter 7).

The lasting effect of a bout of hypoxia on substrate use is less well examined however. In a study from Katayama et al. (2009) an increase in CHO utilisation at rest was observed 60 minutes post an exercise bout in hypoxia again highlighting the preference for CHO use in conditions of reduced oxygen availability. In more recent work however, an increase in the utilisation of fat sources immediately after acute exposure to hypoxia whilst resting in normoxia has been reported (Workman and Bassett 2012). These findings are attributed by the authors of the study to the repayment of an oxygen deficit induced by reduced FiO_2 . Workman and Bassett (2012) postulate that this is most likely through an increased autonomic neuroendocrine stimulation of lipolysis, increased catecholamine release partially responsible for stimulating higher lipid usage, and as a response to greater CHO utilisation during exposure to hypoxia (Workman and Bassett 2012). It is postulated that exposure to hypoxia induces responses that are similar to an exercise induced metabolic perturbation in which a shifting towards greater lipid use following exposure occurs. Also, in contrast to the present study, work from Tonini et al. (2011) observed following a 14 night intermittent hypoxic exposure protocol there was a shift towards greater fat oxidation during exercise in healthy participants, specifically a shift in the maximal lipid utilisation towards a higher exercise workload. It is suggested that these findings occurred independently from changes in insulin resistance and rather a preferential utilisation of lipid and a reduced glucose utilisation in oxidative muscle fibres (O'Donnell, 2007; Tonini et al. 2011).

It has also been proposed, by Sidossis and Wolfe (1996), that contrary to the prediction of the glucose-fatty acid cycle, that intracellular availability of glucose rather than FFA levels is the determining factor for substrate oxidation (Sidossis and Wolfe 1996) and thus increased lipid utilisation may be secondary to reduced glucose disposal in the muscle. Although direct measurement of glucose did not occur in the present study lack of change in CHO oxidation, Insulin and circulating plasma FFA suggests alterations in glucose disposal did not occur. It is also suggested that the findings of a shift in lipid utilisation may be similar to those observed following endurance training (Brooks and Mercier 1994; Tonini et al. 2011). This suggestion is in agreement with the proposed explanation from Workman and Bassett (2012) in which the metabolic perturbations following an acute bout of hypoxia are likened to those following a bout of exercise. Based on these findings it was therefore hypothesised that during a longer term stay at high altitude, during which a greater proportional increase of CHO oxidation was observed, substrate oxidation would be altered upon return to sea-level resulting in a greater oxidation of fat stores in response to a high fat meal when compared to pre-travel responses similarly to an acclimation or training type effect.

Following the ingestion of a high fat load the typical response in lean individuals is to observe increases in fat oxidation and increases in blood lipid profiles including TAGs and, (often following an initial decrease), FFAs (Giacco et al 2003; Blaak et al, 2006). A reduction in plasma glucose coinciding with an initial increase in insulin prior to return to baseline values has also been observed following such a meal (Giacco et al 2003; Blaak et al, 2006). As previously mentioned an increased oxidation of fat upon return to sea-level from high altitude was hypothesised, based on acute work from this thesis

and previous literature (Workman and Basset 2012), and following a time period of increased CHO oxidation during altitude living. Contrary to this hypothesis however, the current investigation observed no alteration in CHO and fat oxidation nor any change in RER values in response to a high fat meal at sea-level following an 18 day stay at high altitude compared to pre-travel values. These findings were observed despite an increase in RMR values highlighting the thermic effect of the meal. These findings demonstrate that the immediate response to acute hypoxia differs to any lasting effect that may occur following extended residence at high altitude. Lack of alteration in substrate use is likely due to the length of time between return to sea-level and the post-altitude assessments. Although lasting effects of altitude stay have been observed upon return to sea-level; namely a lasting reduction in body mass and a lasting elevation in RMR in obese males following seven days at 2,650m four weeks post-return to sea-level (Lippl et al., 2010), in the present study no sustained effects were observed in substrate use. Specifically the mechanisms underpinning the alterations in substrate use observed upon acute exposure have been suggested to include those similar to the observed alteration in post-exercise metabolism.

Increased catecholamine concentration following exercise has been suggested to stimulate higher lipid use which is associated with post-exercise metabolic rate (Laforgia et al., 1997). Adrenocorticotrophic hormone (ACTH) has previously been seen to induce steroidogenesis at both rest and during exercise in hypoxia ultimately increasing lipolytic responsiveness of adipocytes to catecholamines (Laforgia et al., 1997). Therefore alterations in the neuroendocrine system are considered the most likely mechanisms leading to altered post-exposure substrate use. The transient nature of these changes however most likely results in any measurable effects disappearing following one week return to sea-level and thus stand as a reason for a lack of observed change in this study. Another suggested reason for the discrepancy between findings observed following acute, severe and intermittent exposures in previous literature and longer-term exposure in the present investigation is the acclamatory response that occurs during stay at altitude in comparison to the more severe immediate response from a single exposure.

Explanation for the current findings is also likely influenced by the ingestion of a high lipid meal. In previous work from Piers et al. (2002) postprandial fat oxidation was lower when individuals consumed a breakfast high in saturated fat, primarily from a dairy source of cream, compared to a breakfast of the same calories in which the fat source was derived from a mono unsaturated fat of olive oil (Piers et al., 2002). In the present study the contents of the high lipid meal being predominantly derived from cream may be a reason for the lack of alteration in substrate utilisation, particularly fat oxidation. There is also a suggestion from work by Piers et al (2002) that individuals with lower waist circumference have lower fat oxidation following a meal high in saturated fat compared to those with a high waist circumference. In the present study, physically active, healthy weight individuals participated thus being another suggested reason for a lack of change in fat oxidation following the high lipid meal. Acute exposure to simulated hypoxia equivalent to an altitude

of 4,300m has previously shown to induce a preventative effect on postprandial hyperglycaemia (Kelly et al., 2010). Acute exposure to a simulated moderate hypobaric hypoxia equivalent to 2,500m has also been shown to augment carbohydrate oxidation in healthy males without affecting the glucose or insulin response, as demonstrated through an increased RER following a 75 g glucose load compared to that seen in normoxia (Goto et al., 2015). These findings further support the discrepancy between acute responses and longer term lasting responses to altitude.

BLOOD MEASURE RESPONSES

In accordance with findings from study 3 (Chapter 6) in the present thesis a high lipid meal induced increases in plasma FFA although no differences were observed across trials. As previously mentioned these results support the typical response to a high fat meal in normoxia (Giacco et al 2003; Blaak et al, 2006). These findings suggest that an 18 day stay at 3,400m induces no lasting postprandial effects on blood lipid responses following a week at sea-level post-exposure. Exposure to altitude/ hypoxia has provided conflicting results in previous literature with regard to lipid responses and circulating FFAs. Increases in circulating FFA, seen in both rats (Yin et al., 2009) and humans (Jones et al., 1972), are not necessarily representative of an increased rate of lipid oxidation. Accordingly a decrease in the uptake of FFA and glycerol by resting muscle has been reported in conjunction with increased plasma levels of FFA and glycerol following exposure to 4,300m as has an increased dependence on blood glucose for metabolic fuel (Roberts et al., 1996). A decreased reliance on fat metabolism during exposure to altitude is supported by work from Kennedy et al. (2001). Depressed activity of the rate-limiting step in the translocation of FFA into mitochondria, carnitine palmitoyltransferase-I (CPT-I) and in the marker of β -oxidation 3-hydroxyacyl-CoA dehydrogenase (β -HAD) in the heart, liver and muscle of rats following acute and chronic exposure to simulated hypobaric altitude of 4,300m suggests a decreased capacity for fatty acid oxidation and is consistent with findings suggesting a greater reliance on CHO at altitude. Although work from Lippl et al., (2010) and Schobersberger et al., (2003) provide some reference the lasting effect of an altitude stay on blood lipids are less well examined and even more so in response to a high fat load.

Obese men, following a seven day altitude stay at 2,400m, demonstrated a reduction in high density lipoproteins, an increase in low density lipoproteins and a decreasing trend in triglycerides upon return to sea-level one week post-altitude stay (Lippl et al., 2010). These findings are dissimilar to the majority of prior findings (Schobersberger et al., 2003) however and remain to be explained. Other findings from Schobersberger, in which individuals stayed at an altitude of 1,700m for three weeks and were tested seven to 10 days after return to sea-level, included a decreased HOMA index as a marker of insulin resistance and a reduction in glucose concentration at 60 and 90 minutes following an oral glucose tolerance test. These findings were reported in conjunction with unchanged fasting glucose concentrations during acclimation to altitude. These post-altitude stay improvements however were possibly influenced by an exercise programme conducted during the sojourn and may

not be attributable to the altitude alone. The effect of exercise may also serve as an explanation for differences compared to the present study.

Other studies suggesting a correlation between hypoxia and blood lipids have done so in light of greater levels of serum HDL-C in high altitude dwelling populations (Sharma, 1990) and increases in HDL-C observed in a population migrating from lower altitudes to high altitude regions (Aitbaev et al. 1990). The effect of sustained exposure to low altitude has also been shown to correlate linearly up to 1,500m with HDL levels and BMI (Domínguez Coello et al. 2000) suggesting mild altitude may also be effective in positively effecting blood lipids. The presence of higher HDL levels at high altitudes is suggested to originate from changes produced in hepatic tissue due to a “re-adaptation” to periodic altitude ultimately altering lipid oxidation at hepatic level, although further work is required in this area (Domínguez Coello et al. 2000). Decreases in LDL cholesterol have also been reported in conjunction with elevated HDL in healthy individuals seven days post-travel from low altitude to 4,350m (Ferezou et al., 1993). More specifically, postprandial changes of increased CHOL, TAG and phospholipids that occurred in normoxia disappeared following the same meal seven days after travel to 4,350m suggesting a hypoxia induced stimulation of plasma TAG lipolysis at altitude (Ferezou et al., 1993). Mechanistic explanations for altered metabolic responses following an altitude stay remain to be clarified, however other suggestions include hypoxia induced changes in key enzymes involved in fat oxidation and utilisation (Kennedy et al. 2001). The lack of change in blood lipid values in the present study when compared from pre-to post-altitude stay again highlights the lack of lasting effects experienced of the particular altitude stay.

As previously mentioned response to a high fat load in lean individuals in normoxia includes a reduction in plasma glucose values and an initial increase in insulin levels (Giacco et al 2003; Blaak et al, 2006). The main driver for insulin release however is glucose sugar and the increase observed is likely contributed to by the sugar content of the lipid meal. Evidence exists highlighting both the impairment and enhancement of insulin action in response to hypoxia (Larsen et al., 1997). Differences observed between previous studies are confounded by altered study design, exposure protocols, variance in the health status of participants and the inclusion of exercise programmes. Nevertheless insulin concentration has been seen to increase acutely (Brooks et al., 1991) and remain elevated for up to one week (Sawhney et al. 1991) before returning to sea-level values with prolonged exposure up to 21 days (Brooks et al., 1991). Furthermore previous work supports the use of acute (Mackenzie et al., 2011) and intermittent (Mackenzie et al., 2012) exposure to hypoxia coupled with exercise for the improvement of fasting insulin sensitivity in individuals with Type II diabetes. Insulin response and secretion following glucose ingestion during acute hypoxia has been seen to be unchanged when compared to the same ingestion in normoxia (Kelly et al., 2010). These findings were observed in conjunction with a lower glucose response suggesting that a single exposure to a simulated altitude of 4,300m induces increased glucose use.

In contrast an acute bout of hypoxia has evoked an impairment of insulin action during a euglycaemic-hyperinsulinemic clamp in comparison to measures taken in normoxia (Oltmanns et al., 2004) with these contradictory findings suggested to be attributed to differences in timing and duration of exposure. Insulin impairment was also reported following two days of exposure to altitude during euglycaemic-hyperinsulinemic clamps but was improved with more prolonged exposure (seven days) with the mechanistic suggestion of increased plasma cortisol and plasma adrenaline concentrations in part causing the decreased insulin action in the short term (Larsen et al., 1997). It is also previously been seen that adrenaline inhibits peripheral insulin action and insulin release in hypoxia (Braun et al., 2000) although increased adrenaline upon exposure to hypoxia has also been reported independent of changes in insulin (Kelly et al., 2010). Accordingly it is suggested that a 10-fold increase in adrenaline levels is necessary to evoke a detrimental effect on insulin action (Laakso et al., 1992). Furthermore chronic administration of noradrenaline has been seen to increase peripheral insulin action (Lupien et al., 1990). Yet no observed differences in postprandial insulin concentration were observed in the present investigation from PRE to POST1 and POST2, suggesting that an 18 day stay at high altitude induces no lasting effect on insulin action within one to four weeks return to sea-level. The author suggests that any effects of the altitude stay upon insulin action were transient and had been reversed after the initial measure in the present study, seven days after the return to sea-level.

Similarly to contractile activity, hypoxia stimulates glucose disposal in skeletal muscle demonstrated independent of muscle contraction in type II diabetics (Mackenzie et al., 2011). Enhancement of the glucose disposal effects of exercise are observed when combined with moderate hypoxia suggesting a combined effect of the exercise and hypoxia (Mackenzie et al., 2011). Intermittent exercise combined with hypoxia has also been seen to improve HOMA insulin resistance (Mackenzie et al., 2012). Contrary to these findings no alterations in HOMA index as a marker of insulin resistance were observed following the altitude stay. Similarly no changes in HOMA index have also been described acutely following ascent to 2,590m (Stöwhas et al., 2013) and following a seven day stay at 2,650m (Lippl et al., 2010). The characteristics of the sampled population being of a “healthy” weight and diabetic free may explain lack of changes in HOMA index and other markers of health including HDL, LDL in the present study that have been seen to improve following an altitude stay in other investigations (Lippl et al., 2010). The time point of measurement of HOMA at one and four weeks return to sea-level as opposed to at altitude/hypoxia is likely to also contribute to these findings.

LIMITATIONS AND FUTURE DIRECTIONS

Inability to immediately measure the volunteers upon return to sea-level is considered a limitation to the present study. Primary measurement seven days after the final day of altitude stay may have resulted in initial effects being missed however the aim of the present study was to identify effects that may contribute to a meaningful post-exposure effect on metabolic markers and therefore one

week post-exposure was appropriate for the aim. Nevertheless the immediate responses to a longer term altitude stay upon return to sea-level should be examined further in order to more accurately observe any hypoxia induced effects sustained following the stay and to track the time course and decay of any of these effects. Participants lived “freely” during the altitude stay with no strict dietary or activity interventions measured or controlled for. Moreover control of diet and physical activity in the interim period between post-altitude measures was not possible and may have influenced the present results. The recruitment of males and females and in particular the lack of control of the menstrual cycle in females must also be considered a limiting factor to the investigation. Alterations throughout phases of the menstrual cycle likely contributed to altered resting metabolic rate, substrate oxidation and blood lipid responses amongst females. Increased variation within the results must be considered likely in this regard. Furthermore potential differences in the response to altitude in males and females, namely substrate oxidation and RMR result in the potential for a wide variation in results and must be considered throughout the interpretation of the study.

8.6 CONCLUSION

A high lipid drink prior to an altitude stay induced similar effects in blood lipid response as it did following an 18 day stay at 3,400m at one and four weeks return to sea-level. These findings suggest that an intermediate term stay at high altitude induces no lasting postprandial effect on blood lipid response at sea-level and do little to alter substrate oxidation upon return to sea-level from altitude. Specifically; following the ingestion of a high fat load, an intermediate stay at 3,400m provides no effect on substrate oxidation or lipid profiles upon return to sea-level. This is in contrast to previous studies (Ferezou et al., 1993) in which an immediate response to hypoxia was observed to blunt the postprandial blood lipid response. It is therefore likely that a time dependent effect of hypoxia exists with regards to postprandial blood lipid responses. These findings suggest that in previous studies in which exposure to hypoxia has induced lasting reductions in body mass (Lippl et al., 2010) it is unlikely that contribution of different substrates is important for the continued loss of body mass and fat.

9. GENERAL DISCUSSION

9.1 OVERVIEW

The overarching aim of the thesis was to determine both the acute and lasting effects of short term and intermediate length exposure to an environment of reduced oxygen availability, on variables with a potential contribution to changes in body mass. The thesis is drawn to a close through the presentation of a theoretical model in which the effects of altitude on body mass are displayed.

A multidisciplinary approach was taken using laboratory based experiments and field based data collection. The labs were the Welkin site laboratories and the field base was a period of residence at a hypobaric altitude of 3,400m in Peru. Acute and intermediate length exposures were used to probe the effects of reduced atmospheric oxygen on metabolic and physiological characteristics. Consideration of activity was included as the interplay between metabolism, activity and body mass is well recognised. A multifactorial approach to the metabolic responses to altitude was appropriate given the numerous factors that collectively influence body mass. As such the examination of resting metabolic rate, substrate utilisation, appetite, taste and blood lipid responses to a hypoxic exposure provide the basis for the conclusions drawn within this thesis. Furthermore the examination of Meteorin-like following both acute normobaric hypoxia and intermediate hypobaric hypoxia demonstrates novelty in the work presented.

The holistic approach taken throughout the experimental work in the thesis is highlighted most notably through the model presented within this general discussion. The model identifies the individual contribution of a variety of measured variables to reduced body mass upon exposure to reduced environmental oxygen availability. Most notably the relative contributions towards body mass loss of energy expended, energy intake and their influencing factors at altitude is highlighted.

9.2 THESIS AIMS

Previous investigations have highlighted alterations in resting metabolic rate and substrate utilisation following acute and passive exposure to normobaric hypoxia (Workman and Basset, 2012). Within this thesis acute interventions were selected based upon such evidence and as such the initial part of this thesis is centred on the effects of acute exposure to severe hypoxia. Blood lipid responses which provide information regarding substrate metabolism have yet to be examined over a similar acute period of time however. The acute nature of exposure provides information regarding the blood lipid response over a time period more feasible to an intervention strategy. Furthermore, little is known regarding the physiological response to the combination of moderate intensity exercise immediately preceded by normobaric hypoxia. This data will provide information of use as it will allow for the evaluation of physical activity in normoxia during which exercise capacity/work is not hindered coupled with the physiological response to a severe bout of hypoxia.

Longer term investigations lasting for a period of weeks have also examined the effect of an altitude stay on metabolic rate, substrate use, appetite and other measures that contribute to changes in body mass. The effect of an extended altitude stay on taste sensation is unknown however. Data regarding altitude induced reductions in sweet, salt and bitter taste thresholds provide useful information on the contributory factors leading to altered food craving and subsequent food intake. Lasting metabolic effects of the altitude stay were then evaluated upon return to sea-level on two occasions with the latter occurring at one month post-return from altitude. Fasted measures and measures following a high fat meal were taken in order to assess the effect of an altitude stay on postprandial parameters. The final aim of the thesis was to assess the immediate and lasting effects of acute and intermediate length exposure to hypoxia/altitude on levels of plasma Meteorin-like. This novel data will provide information important for contribution to whole body EE and future work examining the effect of extreme environments on the thermogenesis of adipose tissue.

In summary the aim of the current thesis was to examine the immediate and lasting effects of acute and intermediate length exposure to reduced atmospheric oxygen on parameters important to changes in body mass. This general discussion will summarise the principle aims, findings and contribution of each experimental study chapter within the thesis. The practical applications and future research questions arising from the data will then be presented prior to the presentation of a model in which the contributory variables associated with metabolic responses and loss of body mass upon exposure to altitude will be presented. Practical recommendations arising from the thesis will be offered, prior to an acknowledgement of the limitations of the work and a summary of the areas upon which future research can extend.

9.3 PRINCIPLE FINDINGS

Experimental study 1 (Chapter 4) examined the validity and the reproducibility of an online gas analyser (MetaMax 3X) against the gold standard Douglas Bag method during an acute exposure to an environment of normobaric hypoxia ($FiO_2:0.12$). This was conducted in order to assess the use of the MetaMax3X for subsequent studies. The study hypothesised that the MetaMax 3X would provide both reproducible and valid values. The results indicate that the MetaMax 3X has levels of reproducibility that are acceptable and comparable to previous investigations. The study's hypothesis based on reproducibility was therefore accepted. Variability in measures at rest across techniques however necessitates rejection of the validity based hypothesis. MetaMax 3X values of $\dot{V}O_2$ and RER may lead to errors in the estimate of EE by +25 kcal. hr^{-1} and 40% increases in fat utilisation compared to values obtained by the Douglas bag method. These results inform the use of the gold standard Douglas bag method as a measure of indirect calorimetry where possible throughout the remainder of the thesis and provide information with regard to the interpretation of data when collected using the MetaMax3X.

Experimental study 2 (Chapter 5) identified the metabolic effects of acute normobaric hypoxia ($\text{FiO}_2:0.12$) in a group of “healthy” weight individuals. Measures of substrate oxidation, resting metabolic rate and circulating levels of triglycerides and free fatty acids were recorded. Results from the study indicate that a single acute bout of hypoxia is sufficient in increasing EE predominantly through increased utilisation of CHO in agreement with the hypothesis of the study. In addition observed increases in circulating blood plasma FFAs (+ 54%) and TAG (+ 26%) suggest passive exposure to severe hypoxia induce metabolic effects in humans and should be further investigated in conjunction with physical activity.

Experimental study 3 (Chapter 6) quantified the metabolic effects of moderate intensity cycling exercise (60% HRR) immediately preceded by exposure to acute normobaric hypoxia ($\text{FiO}_2:0.12$). The independent and combined effects of acute exposure to hypoxia and moderate intensity exercise were established. In agreement with findings from study 2 (Chapter 5) a 60 minute passive exposure to $\text{FiO}_2:0.12$ evoked increases in EE through increases in both the oxidation of fat and CHO. The primary hypothesis was thus accepted. Increased resting metabolic rate may occur through an increased sympathetic drive (Mawson et al., 2000), and higher basal noradrenaline levels (Urdampilleta et al., 2012). Energy expended and fuel use during exercise was not affected by a prior hypoxic exposure. The secondary hypothesis was therefore rejected. Finally lipaemia following a high fat meal was lower when exercise was undertaken highlighted by reduced TAG concentration but concentrations of MetrnI were unaltered throughout. The results of this study indicate potential metabolic benefits of a short term acute exposure to severe hypoxia however the same exposure may be insufficient to alter MetrnI concentrations and thus exposures of differing lengths and or severity should be examined.

Experimental study 4 (Chapter 7) evaluated the short term and lasting effects of an intermediate length stay (18 days) at moderate altitude (3,400m) on similar metabolic measures examined in studies 2 and 3 (Chapters 5 and 6). Additional measures of body mass, body composition, appetite and taste sensations were also evaluated throughout the investigation. Previous investigations demonstrate reduced body mass upon exposure to hypobaric hypoxia (Lippl et al, 2010). Lasting reductions in body mass were observed up to four weeks post-return to sea-level in conjunction with increased CHO oxidation during the altitude stay. Mean body mass losses of $146 \text{ g}\cdot\text{day}^{-1}$ on average at DAY18 are similar to previous findings (Butterfield 1996; Macdonald et al., 2009). Moderate altitude stay reduced taste sensation of sweet salt and bitter tastes following 12 days of altitude living. No alterations in circulating MetrnI were observed when measured at one and four weeks upon return to sea-level. These results indicate that an intermediate duration at a moderate altitude may serve as an effective weight loss method. Furthermore exposure to moderate altitude highlights a more practical approach when compared to severe levels of hypoxia in terms of acute mountain sickness symptoms. Although no chronic alterations were observed for MetrnI it cannot be ruled out that acute changes may have occurred.

Experimental study 5 (Chapter 8) assessed the lasting effect of the moderate altitude stay examined in study 4(Chapter 7) on the postprandial metabolic effects following the high fat load also examined in study 2 (Chapter 5). A trial prior to the altitude sojourn was compared to two trials upon return to sea-level carried out within one and four weeks. Results of the study indicate that the body's ability to deal with a fat rich meal was unaltered by an intermediate length (18 days) moderate altitude stay when measured upon return to sea-level in both the short (one week) and long (four weeks) term.

TABLE 9.1: Hypotheses for each study chapter presented in this thesis

HYPOTHESIS	ACCEPTED	REJECTED
<u>Study 1 (Chapter 4): Examination of the reproducibility and validity of the MetaMax3X in normobaric hypoxia at rest</u>		
The MetaMax 3X will produce reproducible measures of metabolic markers during exposure on repeated occasions to normobaric hypoxia at rest.	X	
The MetaMax 3X will display good agreement with the gold standard Douglas bag method of collection.		X
<u>Study 2 (Chapter 5): An acute exposure to FiO₂:0.12 induces alterations in markers of lipid metabolism in a healthy human population</u>		
A 60 minute exposure to FiO ₂ :0.12 will increase resting metabolic rate (fuelled predominantly by an increase in CHO)	X	
A 60 minute exposure to FiO ₂ :0.12 will alter resting blood lipid plasma concentrations of FFA and TAG	X	
<u>Study 3 (Chapter 6): Meteorin-like is unaltered by acute hypoxia and subsequent normoxic exercise</u>		
An acute bout of hypoxia will increase resting metabolic rate	X	
An increase in fat through ingestion of a high lipid meal in combination with hypoxia will increase fat oxidation during subsequent exercise		X
The combination of acute hypoxia and moderate intensity exercise will act together to increase the release of Metrnl		X

HYPOTHESIS	ACCEPTED	REJECTED
<u>Study 4 (Chapter 7): Moderate altitude induces metabolic effects in healthy humans acutely and following an 18 day stay at 3,400m</u>		
An 18 day stay at moderate altitude will induce increased RMR throughout the sojourn		X
Acute reductions in taste sensation and lasting reductions in body mass will be induced from an 18 day stay at 3,400 m.	X	
Meteorin-like protein concentrations will increase in response to the altitude stay upon return to sea-level.		X
<u>Study 5 (Chapter 8): The effects of a moderate altitude stay on postprandial metabolic parameters</u>		
An 18 day stay at moderate altitude will improve the postprandial response to a high fat load within one week upon return to sea-level.		X
Any improvement in the postprandial response to a high fat load following an 18 day stay at moderate altitude will cease when measured upon four weeks return to sea-level	X	

9.3 MECHANISTIC OVERVIEW

The following section of this general discussion will outline the suggested mechanisms contributing to changes in body mass upon exposure to altitude or short term hypoxia. Data taken and pooled from across studies within this thesis are presented and discussed in light of previous findings. A model incorporating these mechanisms is then proposed based on the data collected.

9.3.1 RESTING METABOLIC RATE

The effect of altitude exposure on EE is, at present, incompletely defined. Increases (Picon-Reategui, 1961; Butterfield et al., 1992; Reynolds et al., 1999; Lippl et al, 2010), decreases (Nair et al. 1971) and lack of changes (Westerterp et al., 1992; Westerterp et al., 1994) in resting EE have been reported upon exposure to high altitude. When increases are observed, an elevation in BMR through increased sympathetic activation (Butterfield et al., 1992) occurs. However, in the majority of field studies a number of confounding factors including physical activity, cold temperatures, limited palatable food and negative energy balance are present making it difficult to tease out the independent effect of reduced oxygen availability on expended energy. Such factors result in the effect of altitude, alone, on resting EE as unresolved. Furthermore, the contribution of any metabolic changes being attributed to the hypoxia *per se*, independent from other influences, is unknown.

Recent work has addressed the effect of normobaric hypoxic confinement on RMR in an attempt to investigate the isolated effects of reduced O₂ availability (Workman and Bassett, 2012; Mekjavic et al., 2016). An increase in BMR following both an acute (three hours) (+16%) and a short term exposure (mean 12.5% over seven days) at rest eliciting a SpO₂ value of 82% was observed in a group of overweight males (Workman and Bassett, 2012). These findings further support the effect of hypoxia, independent of reduced temperature and increased physical activity, in the alteration of metabolic rate and suggest a correlation between circulatory O₂ transport and BMR in that as O₂ transport is reduced BMR increases. To date, this is the only study to examine the effects of passive, acute hypoxia on responses related to RMR. In the present thesis, pooled data from experimental studies 1, 2 and 3 (Chapters 4, 5 and 6) exposed to FiO₂:0.12 for an acute period, at rest, evoked, on average, an increase in RMR from $1.37 \pm 0.31 \text{ kcal}\cdot\text{min}^{-1}$ to $1.74 \pm 0.23 \text{ kcal}\cdot\text{min}^{-1}$, an increase of $32 \pm 23\%$. This alteration extrapolated to hourly and daily increases of $+22.45 \pm 10.4 \text{ kcal}\cdot\text{hr}^{-1}$ and $+538.72 \pm 249.68 \text{ kcal}\cdot\text{day}^{-1}$ respectively are in agreement with previously stated work from Butterfield, (1992) in which an extra $500 \text{ kcal}\cdot\text{day}^{-1}$ is the suggested requirement in order to maintain body mass at an altitude of 4,300m. Exposure to normobaric hypoxia of FiO₂:0.12 in the present studies correspond to an approximate altitude of 4,500m in line with the work from Butterfield, (1992). Furthermore, these increases are similar to those observed following acute exposure in a study from Workman and Bassett (2012) and are in agreement therefore with the suggestion that hypoxia *per se* is sufficient in inducing a transient increase in RMR in individuals at rest.

Taken together, an increase in RMR and EE in response to exposure to an environment of reduced O₂ availability is most likely induced through an enhanced activity of the sympathetic nervous system (Cutler et al., 2004; Mawson et al., 2000). Exposure to sustained hypoxia of as little as 30 minutes has been shown to be sufficient in increasing sympathetic nervous activity, demonstrated through increases in adrenaline when a gradual decrease of SpO₂ to 75% was administered (Oltmans et al., 2004). In agreement, increases in basal metabolic rate (1392 ± 118 to 1489 ± 134 kcal.day⁻¹), albeit small and transient, and total energy requirement have been reported in conjunction with increased noradrenaline (+ 93%) and sustained elevated noradrenaline levels (+79%) upon exposure to an altitude of 4,300 m in a group of 16 healthy women (Mawson et al., 2000). Observed increases in fasting noradrenaline (1.01 ± 0.03 to 1.30 ± 0.04 nmol.l⁻¹) when “healthy normal weight” males are exposed to an increasing severity of simulated normobaric altitude (2,800 – 3,400 m) for 10 days support these findings for short term exposure (Mekjavic et al., 2016). The pattern of changes in catecholamine response appears to follow that more rapid increases in adrenaline upon exposure occur when compared to increases in noradrenaline responses that occur with a substantial delay (Braun et al., 2001; Oltmans et al., 2004). Although catecholamine measures were not taken in this thesis, mean HR increases of $13 \pm 17\%$ from the same pooled data highlights a possible sympathetic response to the reduced O₂ availability (or from reduced parasympathetic activity). Increases in HR in conjunction with a reduction in SpO₂ ($-15 \pm 6\%$) from the pooled data observed across acute exposure studies in this thesis provide support for this mechanistic suggestion. In the model presented within this general discussion alterations in RMR are suggested to contribute to changes in body mass following exposure to altitude. The replication of similar results in relation to RMR across acute studies in this thesis also provides a body of evidence for this effect. As discussed within the thesis the recruitment of both males and females must be considered with regard to measured changes in metabolism. A greater variation in measured values is likely when both sexes are included in analysis. Furthermore variation is also likely to be increased in female RMR values when tested throughout the menstrual cycle. Differences in EE were observed between the sexes in chapter 7 with greater EE values observed in males (1.36 ± 0.27 kcal.min⁻¹ and 1.06 ± 0.26 kcal.min⁻¹). Although differences exist between the two sexes, increases in metabolic rate upon exposure to high altitude have been seen in females (Mawson et al., 2000) as well as males. Furthermore with the potential application of altitude exposure as a therapeutic tool, both males and females will be of possible benefit therefore supporting the investigation of high altitude exposure on both sexes. Nevertheless the use of a mixed sex population is highlighted within the limitation section of this general discussion.

Although a transient increase in resting BMR and EE is often reported at altitude, it is also observed that an increase in energy requirement with moderate high altitude is not completely explained by a rise in BMR and that other factors are present (Mawson, 2000). A continued maintenance of increased total energy requirement at high altitude (4,300m) compared to sea-level in conjunction with a return of BMR values to sea-level highlights this effect (Mawson, 2000). The following section will initially

outline the effects of acute and intermediate term hypoxia on substrate utilisation within the studies comprising this thesis before suggesting the combined contribution of both RMR and substrate use to loss of body mass.

9.3.2 SUBSTRATE UTILISATION

A change in substrate use upon exposure to environmental hypoxia is commonly observed (Azevedo et al. 1995; Braun 2008; Cartee et al. 1991; Young et al. 1982; Zinker et al. 1994). Substrate partitioning is seen to be altered both acutely (Workman and Basset 2012) and chronically (Brooks et al., 1991) when exposed to altitude in previous work and in this thesis. Data pooled across studies in the present thesis in which an acute bout of hypoxia was experienced; induced a $+0.2 \pm 0.16$ g.min⁻¹ increase in Carbohydrate utilisation and a $+0.05 \pm 0.08$ g.min⁻¹ in fat utilisation. In the same studies CHO contribution increased from 43% to 56% of total EE, following acute exposure resulting in a contribution of 54.60 ± 26.80 kcal.hr⁻¹, an increase of 22.44 ± 12.7 kcal.hr⁻¹ from CHO compared to prior exposure. Furthermore, during the more prolonged stay at altitude in experimental study 4 (Chapter 7) an increase in the proportion of CHO derived fuel was observed in relation to RMR with 1430 ± 344 kcal.day⁻¹ derived from CHO sources at 3,400 m following 18 days compared to 631 ± 295 kcal.day⁻¹ at sea-level. This equated to $38 \pm 17\%$ of total expended energy at sea-level compared to $79 \pm 15\%$ contribution at 3,400 m. The observed effects of acute hypoxia on RMR and substrate utilisation in this thesis culminate in an increased RMR predominantly driven by an increased utilisation of CHO derived sources.

Induced hyperventilation upon initial exposure to hypoxia may be suggested as a possible cause for the observed alteration in RER values and subsequent effects on calculation of increased CHO use. Although values must be viewed with caution, particularly when comparing acute vs chronic findings, this suggestion can be countered (although not ruled out) from data collected in study 4 (Chapter 7) of this thesis. In this study an increased reliance on CHO sources was measured following 18 days of altitude living and thus unrelated to an acute hyperventilation response. Furthermore increased measured $\dot{V}O_2$ during acute exposure provides evidence for a greater energy expended. Moreover the participants had achieved a steady state during exposure and thus the rate of CO₂ excretion and oxygen uptake would be at a new equilibrium. This effect of CHO reliance is supported by previous literature as described extensively within this thesis. Increased reliance on CHO fuels recorded in study 3 (Chapter 6) is supported by \dot{V}_E and $\dot{V}O_2$ values displayed within the study chapter.

In the field setting, increased BMR and alterations in fuel use may be a consequence of not only the hypoxia, but also altitude induced changes in appetite and energy intake (Braun 2008; Butterfield 1996). As such it is often difficult to distinguish between the effects of hypoxia per se and the effects of a negative energy balance on substrate use. It is likely therefore that a number of the reported responses at altitude are an amalgamation of malnutrition coupled with altitude rather than altitude alone (Brooks, 2014). Moreover confounding factors of physical exertion, changes in environmental

temperature and the length and severity of exposure are also present (Butterfield 1996; Young et al. 1982). Nevertheless, the previous literature suggests that a relative contribution of glycolytic versus oxidative ATP production is increased when humans are exposed to hypoxia, further exaggerated with physical exertion (Horscroft and Murray 2014). Specifically, increases in the utilisation of carbohydrate derived fuel sources of glucose, glycogen and lactate are observed when measured at altitude, when energy needs are met by diet in men (Brooks et al., 1991; Braum et al., 2000; Roberts et al., 1996) and in experimental models including isolated muscle (Azevedo et al., 1995), whole rat models (Cartee et al., 1991) and exercising dogs (Zinker et al., 1994) as measured by indirect calorimetry, stable isotope tracers and mass balance measurements for metabolites and tracers (Brooks, 2014). The acute nature of exposure in the present thesis during which individuals sat passively supports the independent effect of hypoxia in increasing CHO contribution to EE. This data adds to the previous literature by observing these changes following a 60 minute bout at $FiO_2:0.12$.

It is proposed by McClelland (2004) that CHO use is more economical in an environment of reduced oxygen availability due to a greater yield of ATP per litre of O_2 consumption when compared to fat, thereby maximising ATP yield per unit of O_2 . Accordingly, when measured at rest and during exercise at 4,300m, CHO, specifically glucose, is the predominant fuel source when individuals are fed to meet energetic needs (Barnholt et al,2006).

Enhanced glucose uptake by skeletal muscle may account for increased CHO oxidation under hypoxic conditions. Non-insulin dependent recruitment of GLUT4 induced by hypoxia is a mechanistic explanation of these observations. Hypoxia itself stimulates GLUT4 mediated glucose transport via several signalling pathways (Constable et al 1988; Ploug et al: 1987; Cartee et al: 1991; Young et al. 1994; Fujii 2006). The translocation of GLUT4 transporters to the cell membrane in response to hypoxia facilitates glucose transport into muscle. Although not the primary response; a second mechanistic explanation for the shift in substrate use include an upregulation of HIF-1 alpha (Semenza et al., 1994) and increased circulating adrenaline levels (Braun, 2008) upon exposure to hypoxia. This pathway however will require an extended time period at altitude for the alteration of gene expression by HIF-1 alpha to occur and subsequently be effective at the whole cell or organism level. This observed shift in substrate metabolism through the HIF-1 alpha mechanism contributes to the commonly observed increase in BMR by creating an “energy wasting cycle” it is proposed (Palmer and Clegg, 2014) highlighting the interplay between metabolic rate and substrate use at altitude. Upregulation in HIF activity has been suggested to contribute to energy wasting and subsequent progressive weight loss in cancer patients. This is based on findings in which Cori cycle activity and glucose production are greater in those patients experiencing progressive weight loss compared to weight stable patients (Young, 1977). Increased Cori cycle activity has been proposed to account for $300 \text{ kcal}\cdot\text{day}^{-1}$ of additional energy loss with these effects occurring upon exposure to altitude and increased HIF activity (Palmer and Clegg 2014).

Finally the normal shift CHO use during exercise could be limited at altitude by the availability of fuel sources if individuals are in a negative energy balance commonly observed at altitude resulting in the forced catabolism of lipid and even lean tissue culminating in loss of body mass if stay at altitude is sustained.

Body mass loss upon exposure to altitude is common. An intermediate length exposure to 3,400 m in this thesis induced a loss of body mass of $146 \text{ g}\cdot\text{day}^{-1}$ which is similar to previous findings of $200 \text{ g}\cdot\text{day}^{-1}$ (Butterfield 1996) and $110 \text{ g}\cdot\text{day}^{-1}$ (Macdonald et al., 2009). An observed increase in EE is a contributing factor to changes in body mass at altitude (Palmer and Clegg, 2014). As such, pooled data taken from the 3 acute studies presented in this thesis in which individuals were exposed to $\text{FiO}_2:0.12$ at rest evoked, on average, an increase of $32 \pm 23\%$ in RMR. A sustained increase of this magnitude extrapolates to a $+538.72 \pm 249.68 \text{ kcal}\cdot\text{day}^{-1}$ in agreement with previous work from Butterfield, (1992) in which an extra $500 \text{ kcal}\cdot\text{day}^{-1}$ is the suggested requirement in order to maintain body mass at an altitude of 4,300 m. Furthermore, an increase of this magnitude, if sustained, would extrapolate to an extra utilisation of $135 \text{ g}\cdot\text{day}^{-1}$ of CHO and $60 \text{ g}\cdot\text{day}^{-1}$ of fat. However, these results must be viewed with caution as adaptive alterations may occur with prolonged exposure.

Increases in RMR during exposure to hypoxia are derived predominantly from an increased use of CHO. These findings support the previously described metabolic shift at altitude towards greater utilisation of CHO derived sources. In agreement with previous work from Workman and Bassett (2012), a greater proportional increase in fat oxidation in relation to calories was observed post-exposure during moderate intensity exercise in chapter 6. These findings suggest a lasting effect of short term exposure that may translate to beneficial responses in EE and fat utilisation as well as highlighting the general metabolic perturbation from acute, severe hypoxia.

9.3.4 BLOOD LIPID RESPONSES

The effect of reduced O_2 availability on blood lipid concentrations, including FFA appearance and uptake is complex and dependent upon, amongst other factors, the energy intake and energy balance of individuals (Barnholt et al., 2006; Roberts et al., 1996). Free fatty acids are the primary oxidative fuel for resting skeletal muscle with a contribution of more than 70% of total body EE following an overnight fast (Boden and Shulman, 2002). Following the stimulation of adipose tissue lipolysis, such as during conditions of starvation and exercise, availability of FFA is increased through the breakdown of TAG in response to sympathetic stimulation via the activation of hormone sensitive lipase (HSL) to release glycerol and free fatty acids (FFA).

In the present thesis it was therefore hypothesised that increased sympathetic activation upon exposure to hypoxia may increase the availability of FFA resulting in a greater oxidation of fat and contribution from fat to increased EE when exposure is coupled with physical activity. Indeed hypoxia alone, and when coupled with exercise, has been shown to raise lipid oxidation (Wiesner et al., 2009)

and the combination of exercise and hypoxia has also induced reductions in fasting TAG concentrations in lean healthy men measured following an eight week training period during which individuals exercised three times per week at 60% of their $\dot{V}O_2$ max, whereas training alone had no such effect (Netzer et al., 2008). Following an acute exposure to severe hypoxia, increases in FFA have been reported in the present thesis (Chapter 5 and 6). Although moderate intensity exercise, used in experimental study 3 (Chapter 6) was effective at lowering postprandial hypertriglyceridemia measured as the area under the curve, in contrast to the study's hypothesis, there was no independent effect of hypoxia on TAG concentration.

Previously, increases in circulating FFA, during chronic stay at altitude have been observed in both rats (Yin et al., 2009) and humans (Jones et al., 1972). Interestingly, a reduction in glycerol release and the uptake of FFA to essentially zero by resting muscle has also been reported when plasma levels of FFA and glycerol are increased following exposure to 4,300 m in conjunction with increased dependence on blood glucose for metabolic fuel (Roberts et al., 1996). These findings support the suggestion that an increased plasma FFA level does not necessarily translate to increased utilisation of FFA. Indeed concentrations alone do not allow for the interpretation of mechanisms, as increases in levels can be brought about by either a greater release or a reduced uptake. When measured upon return to sea-level, in study 4 (Chapter 7), no effect of an 18 day stay at 3,400m was observed on values of FFA and TAG when measured upon return to sea-level. Drawing conclusions on the contribution of lipolysis to reduced body mass upon exposure to reduced O_2 availability is challenging and is affected by a number of confounders including the length and severity of exposure. Previous literature has observed a correlation between increased plasma and liver TAG levels with increased severity of FiO_2 , largely driven by decreased tissue uptake of TAG and decreased lipoprotein clearance (Jun et al., 2012). However, based on the data in this thesis, there is insufficient evidence to conclude that an altitude induced increase in fat oxidation, as measured contributes meaningfully to loss of body mass at altitude.

9.3.5 METEORIN-LIKE

It is suggested that *Metrn1* stimulates both the expression of genes associated with beige fat thermogenesis, and the immune cell subtypes to enter adipose tissue, thus activating pro-thermogenic actions (Rao et al., 2014). Increases in metabolic rate five days post-adenovirus induced overexpression of *Metrn1* have been observed in obese, diabetic mice with no changes in physical activity or alterations in RER (Rao et al., 2014). Previous work has demonstrated that both acute exposure to the cold and physical activity increases *Metrn1* expression in mice (Rao et al., 2014). An improvement in glucose tolerance coupled with increases in EE has previously been observed in humans with metabolic syndrome following a three week stay at 1,700 m (Schobersberger et al., 2003). Although a rationale for an altitude induced change in Meteorin-like is presented within this thesis it may also be argued that hypoxia has the potential to oppose the effects of Meteorin-like. Specifically; a blunting effect of hypoxia to the thermogenic response in cold conditions exists (Blatteis

& Lutherer 1976; Gautier et al. 1991). There is currently no information on the effects of altitude exposure on the responses of Metrnl in humans. These findings serve as a rationale for the investigation of Metrnl responses to both acute and intermediate altitude/ hypoxia exposure.

No acute effects of exercise, hypoxia or fat ingestion on plasma concentration of Metrnl were observed in work conducted in this thesis (Study 3/ Chapter 6). Similarly, a sustained stay at 3,400m induced no lasting effect on concentrations of Metrnl when measured upon return to sea-level within seven days (Study 4/ Chapter 7). Lack of alterations in Metrnl seen in this thesis may be attributed to both the physiological response of the specified altitude and hypoxic stimuli and to factors associated with experimental design, particularly the acute nature of exposure in studies 2 and 3 (Chapters 5 and 6) and the measurement upon return to sea-level following an 18 day stay at altitude rather than measurement throughout the altitude stay in study 4 (Chapter 7). Therefore, it cannot be concluded from work conducted in this thesis that any increased expression of Metrnl contributed to the observed changes in body mass (study 4/ Chapter 7) and that loss of body mass occurs independently of change in Metrnl.

The mRNA expression and secretion of Metrnl is stimulated by the expression of a novel form of PGC-1 α (Ruas et al., 2012). PGC-1 α is a transcriptional coactivator that controls the genes that contribute to oxidative metabolism and mitochondrial biogenesis (Ruas et al., 2012). Increased expressions of mitochondrial UCP1 and PGC1 α in subcutaneous adipose tissue following a long term intermittent cold exposure protocol have been recorded in conjunction with improved glucose tolerance, enhanced insulin sensitivity and reduced weights of epididymal and retroperitoneal adipose tissue in mice (Wang et al., 2015b), thus highlighting the possible link between UCP1 and Metrnl. An increased expression of UCP1 contributes to the “browning” of white adipose tissue resulting in a more brown like tissue that is capable of thermogenesis (Warner and Mittag 2015).

As outlined in the future directions section of this thesis, continued work regarding the response of Metrnl at altitude would benefit from focusing on potential changes associated with longer term exposure or a long term intermittent protocol as these may be necessary to expect an increase in Metrnl compared to the acute exposure investigated in this thesis. Prolonged or repeated exposure to hypoxia may result in an increase in mRNA for Metrnl and thus increased transcription. Furthermore, the measuring of Metrnl whilst resident at altitude would allow for an immediate representation of the effects of altitude stay on Metrnl and allow for the evaluation of the changes, if any, over time spent at altitude. Inability to sample and measure at altitude and the time frame of exposure are therefore potential reasons for the absence of changes observed in the present thesis.

9.3.6 APPETITE, TASTE AND SUBSEQUENT EFFECTS OF ENERGY INTAKE AND ENERGY BALANCE

It has been observed that a reduction in energy intake of 200 kcal.day⁻¹ upon sustained exposure to high altitude is common (Butterfield et al. 1992). This diminished energy intake and a partnering reduction in reported appetite have been observed in both chamber studies (Westerterp-Plantenga

1999; Mekjavic et al., 2016) and studies in which individuals travel to altitude (Bailey et al., 2000; Barnholt et al., 2006; Kalson et al., 2010; Westerterp et al., 2001). A role for hypoxia per se on energy intake, independent of cofounders associated with high altitude travel including the availability and palatability of food, is therefore supported. The exact mechanisms for altered appetite upon altitude exposure however are unclear.

Loss of body mass was observed in the present thesis when the volunteers lived at altitude. A possible explanation for these findings is in part centred upon a reduction in food craving which may have contributed to a reduced energy intake and thus to the measured loss of body mass. Previous studies have reported that changes in craving can alter the drive to eat with no change in sensation of hunger (Geer et al., 2016), thus explaining unaltered appetite ratings in the present thesis. Food cravings of sweet, salt, and bitter tastes have been attenuated during high altitude (2,616-4,200 m) residency (Yan et al., 2011) and are therefore possible mediators in this response. The reduction in sensation of these three tastes following 12 days of altitude living measured in this thesis support a role for a reduced food intake mediated by reduced food craving due to alteration in taste.

Taste is an important regulator of food intake and feelings of satiety and palatability. A loss or reduction in taste has been suggested to directly lead to weight loss (Woschnagg et al., 2002). Specifically, the author suggests a reduction in sensation of taste may result in an increased monotony of flavour and thus, a reduced palatability and pleasantness of food culminating in earlier satiety and reduced energy intake. This is in part, based on the suggestion that body weight maintenance may depend to some extent on the availability of a varied and palatable diet (Rolls, 1995a, 1995b). In fact, previous work indicates that repeated presentation of some foods can lead to a persistent decrease in the pleasantness of the presented foods (Schutz and Pilgrim 1958; Siegel and Pilgrim 1958) and the consumption of a monotonous liquid diet, was found to cause subjects to voluntarily restrict their energy intake and thus lose weight (Cabanac and Rabe 1976). Furthermore, when freely available diets are varied and palatable, subjects show excessive weight gain (Porikos et al. 1977; Porikos et al. 1982).

These previous findings provide support for a reduction in energy intake mediated by the pathway of reduced sensation of taste. Data in this thesis show a reduction in sensation of taste thresholds in salt, sweet, and bitter sensations which may contribute to altered food choices and thus, reduced energy intake during intermediate exposure to altitude (3,400 m). Accordingly, correlations exist between taste perception and anorexia, decreased food intake and weight loss (Ames et al., 1993; Mattes and Cowart, 1994; Dewys and Walters, 2006 in Chapman-Novakofski et al., 1999). In patients with cancer it is shown that those who demonstrate greater loss of body mass also show a more frequent occurrence of changes in taste sensation (Grosvenor et al., 1989). Taken together, reductions in sensation of taste in the present thesis may be a contributing factor to the observed loss of body mass

observed through a reduced motivation to eat due to a reduction in pleasantness of taste leading to altered food preferences and/or reduced intake.

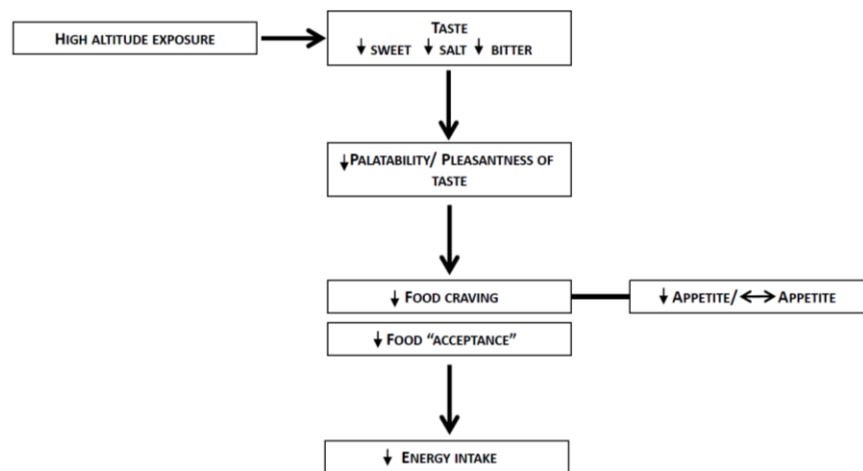


Figure 9.1: Illustration of the influence of reductions in sensation of taste on subsequent energy intake.

Notes: ↓ represents a decrease in a measure. ↔ represents no change in a measure

Similar to suggested alterations in RMR and substrate utilisation; previously described hypoxia induced increases in sympathetic activity, may be mechanistic contributors to reduced appetite upon ascent / exposure due to the appetite depressant effects of noradrenaline (Hainer et al., 2006). Furthermore, changes in appetite regulating gut hormones upon both acute and prolonged exposure have also been seen to be altered and may play a role in energy intake at altitude (Scherer, 2006; Smith et al., 2011; Kayser and Verges 2013) although current research is equivocal (Tschöp et al. 1998) with both increases and decreases of appetite suppressing hormones present in the literature. Interestingly, the combination of both continuous moderate and high intensity interval training in normobaric exposure has been seen to induce reductions in appetite perceptions and plasma acylated Ghrelin in response to as little as 50 minutes normobaric hypoxic exposure whilst performing exercise (Bailey et al., 2015). Similarly, recent work from Debevec et al., (2014) observed reduced energy intake following a 10 day confinement in individuals performing regular physical activity when compared to pre-confinement values, although no change in appetite ratings were seen, suggesting a possible reduction in motivation to eat when exercise and hypoxia are combined. These findings suggest that a combination of hypoxia and exercise, similar to that examined in experimental study 3 (Chapter 6), may be a potential method to induce adaptations useful for reduced energy intake and thus, loss of body mass.

Reduced energy intake at altitude is a contributing factor to an overall negative energy balance upon exposure. Alterations in energy balance have been linked with the previously discussed changes in

RMR, further highlighting the interplay between various contributing factors to weight loss at altitude. Recent work, has suggested that an initial energy deficit at altitude may be a contributing factor in the transient increases in BMR (Mawson et al., 2000). Individuals described as “non-eaters” (participants that were unable to eat adequately upon exposure to meet their EE) in a study from Mawson et al., (2000) demonstrate a greater increase in BMR when compared to “eaters”. Other work has reported increases in BMR in conjunction with an energy deficit. Specifically a 28% increase in BMR in women experiencing an energy deficit of 2930 kJ.day⁻¹ below sea-level intake was observed (Hannon and Sudman 1973) as was a similar increase in men who were fed ad libitum, yet consumed less than required to maintain energy balance (Kellogg et al., 1957; Moore et al., 1987 in Mawson et al., 2000). These findings support the suggestion that upon exposure to altitude an increase in metabolic rate, coupled with, and perhaps driven by negative energy balance, culminate in loss of body mass. This negative energy balance, in part due to reduced energy intake, is possibly caused by changes in taste sensations and increased catecholamine release upon exposure to reduced O₂ availability.

9.4 PRACTICAL APPLICATION OF FINDINGS

The studies conducted within this thesis were intended to contribute to a multi-factorial, mechanistic explanation of changes in body mass and metabolism in humans upon exposure to conditions of reduced O₂ availability. The level of normobaric hypoxia adopted in experimental studies 1, 2, and 3 (Chapters 4, 5 and 6) and the level of hypobaric altitude experienced in experimental studies 4 and 5 (Chapters 7 and 8) are both considered likely to elicit the physiological responses of “high altitude” (Bärtsch and Saltin 2008). Consequently, these physiological responses reported throughout the thesis may serve as a useful index for both the acute and intermediate length effects of high altitude on metabolism and the contributing factors to changes in body mass. While the aims of the thesis are centred upon a mechanistic approach some practical applications can be drawn from the collected data. Namely findings from the thesis may have application for the experimenter using online gas analysis as a method of indirect calorimetry in an environment of normobaric hypoxia. Specifically, the data has provided values that will allow for the comparison of results with the gold standard method and provide % values of overestimations observed. Results from the thesis may also have relevance and practicality for the growing number of individuals travelling to high altitude aiming to maintain body mass. Specifically the quantification of an observed increase in EE experienced from an acute bout of hypoxia has been reported and replicated. Furthermore, support for the observed loss of body mass following an intermediate length exposure to high altitude is reported. Moreover, relevance of data collected regarding the use of acute altitude exposure in isolation or in combination with moderate intensity exercise may serve to inform the use of hypoxia as a tool to aid losses of body mass and alterations in important factors of metabolic health. Taken together, the data collected throughout the thesis will inform future research investigating similar effects of reduced O₂ availability on metabolism in man.

More specifically, the practical importance of experimental study 1 (Chapter 4) is centred on the use of a commonly used cardio-metabolic analyser in an environment of normobaric hypoxia. The quantification of variance in measures of $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER between repeated uses of the MetaMax3X and in the comparison between the MetaMax3X and the gold standard Douglas bag method supply practical considerations for the interpretation of collected data. The knowledge of such differences in important measures will increase confidence in the interpretation of data and may also result in an appropriate level of caution exercised in the conclusions drawn when based on such data. Derivative measures based on the values of $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E and RER will also be affected by differences observed across trials and between methods as discussed in Chapter 4. Such measures include, within this thesis, RMR and the oxidation of CHO and fat. The awareness of possible differences across methods has the potential inform future research in relation to study design and data interpretation.

With a large number of individuals travelling to high altitude for leisure or adventure activities it is therefore true that increasing numbers of individuals are exposed to its accompanying physiological effects (Schobersberger et al., 2003). As discussed throughout the thesis, particularly within experimental study 4 (Chapter 7) and within section “Loss of body mass and alterations in metabolic rate at altitude” of the literature review (Chapter 2), loss of body mass is a common consequence of this type of travel particularly when coupled with physical exertion such as mountaineering. Findings from this thesis, particularly data from experimental study 4 (Chapter 7) has the potential to inform the magnitude of body mass change that will occur during a period of “free-living” at an altitude of 3,400 m. This is useful as it will inform nutritional strategies for those aiming to maintain body mass upon ascent, such as for those mountaineering. This data is also of use as a tool to calculate the likely loss of body mass over a given time period at high altitude for individuals aiming to lose body mass. Data collected in this study will also inform the likely changes in substrate use both during and following a sojourn to high altitude.

These findings may be helpful in assisting in the development of methods and interventions to minimise the unwanted loss of body mass in individuals whilst at high altitude. A loss of body mass, if derived from fat free mass, may result in a loss of functionality and strength which may therefore culminate in detrimental performance and ability to carry out everyday tasks. Following an altitude stay in the present thesis, body composition changes were not compartmentalised to a greater ratio of fat or muscle demonstrated by the analysis of TBW, BF and LM through multi-frequency impedance. Although the primary aims of the collection of studies presented in this thesis was not to investigate the loss of fat free mass upon exposure to altitude the data presented may go some way in informing future research with regards to likely losses of mass and may also inform dietary choices of the traveller at altitude.

The collection of data following return to sea-level after a period at high altitude in experimental studies 4 and 5 (Chapters 7 and 8) provides information on the short term lasting effects of hypobaric conditions on body mass, the blood lipid responses to a high fat meal, RMR and substrate use. Practically, this data may inform the use of altitude exposure in the context of an intervention that incorporates repeated exposures to hypoxia as a tool for potential improvements in metabolic parameters by informing the time gap between repeated bouts. Specifically, no observed effects on measures of FFA, TAG, Insulin and substrate use at both one and four weeks upon return to sea-level following a high fat meal highlight an unaltered ability to deal with a fat rich meal at sea-level.

The use of reduced O₂ partial pressures by using environmental chambers and “true” altitude has recently been suggested to have potential benefits for health. Specifically, the use of hypoxia as a method of body mass loss has been shown in obese participants exposed to a hypobaric environment (Lippl et al, 2010) and a normobaric hypoxic environment coupled with aerobic exercise (Netzer et al, 2008; Wiesner et al, 2010). Data from this thesis, specifically experimental study’s 2 and 3 (Chapters 5 and 6), support the notion exhibited by previous findings that hypoxia may have potential beneficial effects in variables associated with body mass. Alterations in RMR from acute exposure may have practical application in altering the energy balance of individuals in need of loss of body mass. Whether the observed alterations in RMR are sufficient to result in a meaningful difference in body mass however is undetermined from the current data. These findings, nevertheless, have the potential to inform future research and intervention strategies on the use of short-term exposure to severe hypoxia. This thesis also demonstrates that the addition of a moderate exercise bout to an acute passive exposure to severe normobaric hypoxia does not immediately provide an additive benefit in relation to EE although the same moderate exercise bout was sufficient in reducing postprandial plasma TAGs.

The use of reduced O₂ partial pressures by using environmental chambers and “true” altitude has recently been suggested to have potential benefits for health. Specifically, the use of hypoxia as a method of weight loss has been shown in obese participants exposed to a hypobaric environment (Lippl et al, 2010) and a normobaric hypoxic environment coupled with aerobic exercise (Netzer et al, 2008; Wiesner et al, 2010). Data from this thesis, specifically experimental studies 2 and 3 (Chapters 5 and 6), support the notion exhibited by previous findings that hypoxia may have potential beneficial effects in variables associated with body mass. Alterations in RMR from acute exposure may have practical application in altering the energy balance of individuals in need of loss of body mass. Whether the observed alterations in RMR are sufficient to result in a meaningful difference in body mass however is undetermined from the current data. These findings, nevertheless, have the potential to inform future research and intervention strategies on the use of acute exposure to severe hypoxia. This thesis also demonstrates that the addition of a moderate exercise bout to an acute passive exposure to severe normobaric hypoxia does not immediately provide an additive benefit in relation to EE although the same moderate exercise bout was sufficient in reducing postprandial plasma TAGs.

The work conducted and presented in this thesis culminates in the presentation of a model illustrating contributing factors to changes in body mass upon exposure to altitude (Figure 9.1). The effect of altitude on both energy intake and EE is displayed. The novel assessment of MetrnI following altitude exposure is exhibited as is the suggested contributory effect of taste changes at altitude. Alterations in taste upon exposure are suggested to contribute to reduced energy intake through a reduction in food craving which is illustrated in the model. From data collected throughout this thesis and supported by previous work an approximate contribution of 111 g.day⁻¹ to the 146 g.day⁻¹ body mass losses observed at high altitude is explained by an increase in EE. This accounts for approximately 74% of the observed loss. An approximate 22-29 g.day⁻¹ is accounted for by the reduction in energy intake a contribution of between 15 – 20% of total body mass loss. Taken together the model provides a theoretical suggestion of contributors to loss of body mass based upon data collected in this thesis and previous work within the field of high altitude research.

Although not illustrated in the proposed model the use of physical activity in combination with exposure to hypoxia was examined in this thesis. A 60 minute cycling exercise bout at a moderate intensity (60% HRR) induced a protective effect on postprandial hypertriglyceridaemia. The exercise bout induced a kcal expenditure of $+7.22 \pm 1.97$ kcal.min⁻¹ compared to resting measures equating to $+433 \pm 118$ kcal over a 60 minute bout. Based on this data and based on the relative contribution of fat and CHO it is proposed that an exercise bout of a similar nature would contribute a further 101 g.day⁻¹ to loss of body mass. This is however a speculative suggestion based on data collected within this thesis. Due to the complexity of energy balance and its contributing factors this suggestion should be viewed as a starting point for future research and thus must be treated with caution. As outlined in the future direction section of this discussion further work is required in order to better understand the effect of physical activity and hypoxia combined.

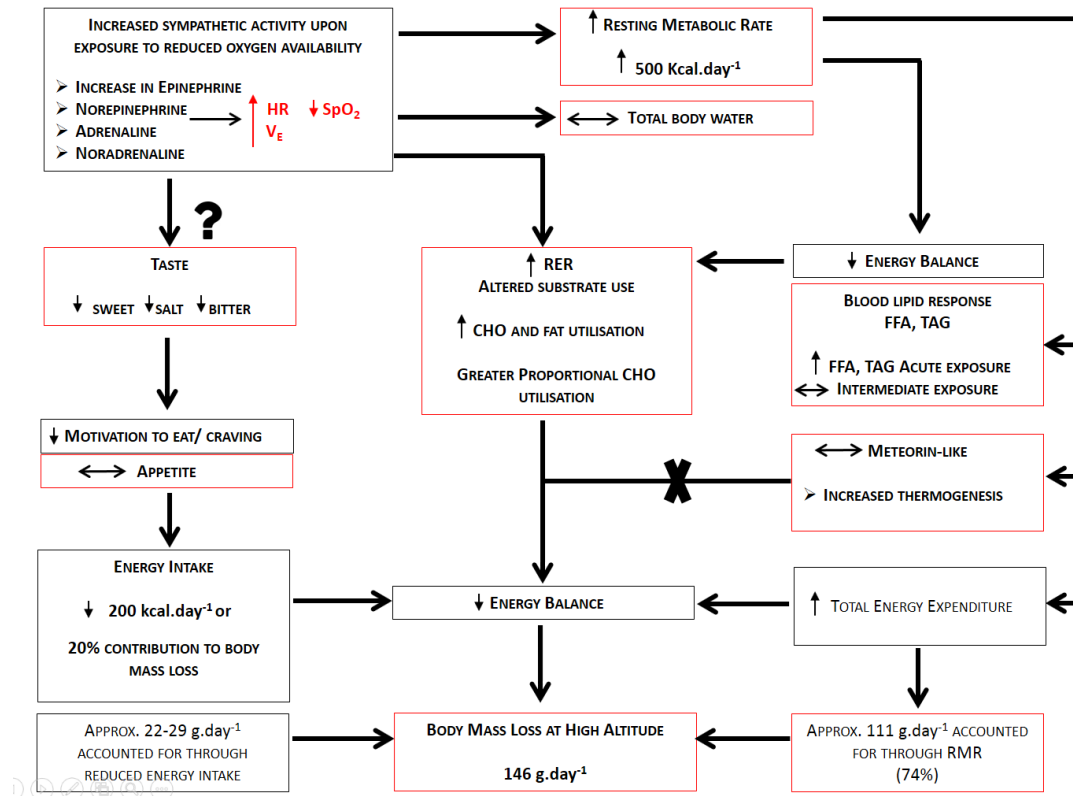


Figure 9.2: Proposed theoretical model of the determining factors of reduced body mass upon exposure to high altitude.

Notes: The physiological determinants of body mass loss encircled in red represent measures taken within this thesis. Physiological measures in this model will directly influence the magnitude of body mass loss upon elevation to high altitude.

The current thesis has outlined the potential benefits of hypoxia and used these as the basis for further investigation into therapeutic effects in an unhealthy population. Although the rationale for this investigation is sound, the potential negative effects of altitude/hypoxia must also be recognised. Increased sympathetic nervous activity upon exposure to hypoxia, as previously mentioned, induces a number of physiological changes in humans. Increased arterial blood pressure, upon acute exposure to altitude is one such observation (87 ± 3 at 5,260m vs. 77 ± 2 mmHg at sea-level) (Hansen and Sander, 2003). Increased blood pressure upon exposure, particularly in individuals with an already raised blood pressure, is therefore a considerable risk that must be investigated further prior to long term programmes incorporating exercise and hypoxia being advised.

Reduced immune function upon altitude exposure has also been outlined as a potential negative effect of hypoxia. Hypoxia is described as an inducer of inflammation and of immunosuppressive agents (Caris et al., 2017). Increased resting measures of adrenaline, cortisol and interleukin-6 at high altitude (Mazzeo et al., 2001) support the hypothesis that physical activity at altitude may pose a

greater challenge to immune function than that at sea-level (Svendsen et al., 2016). Increases in neutrophils and lymphocytes, cell proliferation and natural killer cells are reported (Walsh and Oliver, 2016). Furthermore impairment in immunity due to changes in the number and function of T lymphocyte cells is observed with in-vivo and in-vitro cell-mediated immunity decreased during exercise at altitude and self-reported upper-respiratory tract infection increased at altitude (Facco et al., 2005; Oliver et al., 2013). Interestingly however, it is also hypothesised that exercise and certain supplementation may have potentially positive effects on mitigating the negative effects of hypoxia on immunosuppression although further investigation is required in this regard (Caris et al., 2017). Although knowledge of alterations in immunity at high altitude is not complete, this issue must be considered during the future investigation and implementation of exposure to hypoxia and physical activity as a therapeutic tool.

It is argued that combining physical activity and hypoxia may also be beneficial for unhealthy populations due to the maintenance of relative exercise intensity with a reduced mechanical load from lower absolute intensity (Netzer et al, 2008; Wiesner et al., 2010). Despite this, the additive effect of hypoxia and physical activity must be used with caution with potential risks considered. Severity of hypoxia, length of exposure, rate of ascent and physical activity during exposure must also be thoroughly investigated prior to any programme being administered. Although previous studies have observed unhealthy individuals to tolerate exposure to hypoxia, both alone and combined with physical activity, without reported complication (Schoesberger et al. 2003; Netzer et al, 2008; Lippl et al. 2010; Wiesner et al., 2010) future studies should consider safety with paramount importance. This is of particular importance with long term exposure and with exposure to high altitudes/ severe hypoxia.

Also a point of caution with regard to altitude exposure is the potential increase in preference for sweet tasting food as previously mentioned (Singh et al., 1997, 1996). Although appetite and energy intake is often reported to be reduced upon exposure in field (Bailey et al., 2000; Barnholt et al., 2006; Kalson et al., 2010; Westerterp, 2001) and laboratory studies (Westerterp-Plantenga, 1999) an increased palatability of sweet tasting substances may result in an increased energy intake, particularly during acute or intermittent exposures that may be utilised in therapeutic programmes.

9.5 LIMITATIONS

Despite utmost caution being taken to ensure valid and robust measures throughout all experimental chapters, the findings of this thesis should be considered in light of the following primary limitations:

- Although indirect calorimetry is a suitable and valid measure of substrate oxidation (Jeukendrup and Wallis, 2005) a possible hypoxic induced increase in ventilation upon exposure may lead to an alteration in $\dot{V}CO_2$ and thus values of fat and CHO oxidation. All \dot{V}_E and $\dot{V}CO_2$ data is presented in conjunction with substrate oxidation throughout this thesis however aiding the interpretation of data.
- Although a mechanistic approach was the focus of the studies within this thesis the use of healthy weight individuals could be considered a limitation when suggesting effects of weight loss for an overweight population. As such it has been attempted, during discussion within this thesis, to focus on the mechanistic findings and less so on the implication of these findings to an overweight or unhealthy group. The mechanistic findings in the current thesis will serve as a base for future investigation in different populations.
- In experimental study 1 (Chapter 4) a lack of simultaneous measurement of expired air using both the Douglas bag and MetaMax3X is a limitation of the study and results should be viewed in light of this. However sessions were matched for time of day and sessions were separated by one week in order to avoid any lasting effects of an acute exposure to normobaric hypoxia. Furthermore protocols of a similar nature (Perkins et al. 2004) have been sufficient in providing levels of agreement that are satisfactory in terms of levels of agreement.
- In experimental study 2 (Chapter 5) separate experimental groups for each condition may be considered an influencing limitation to the described work.
- Throughout experimental study 4 (Chapter 7) a lack of dietary data could be considered a limitation and cannot be excluded as a possible cause for loss of body mass during the sojourn. Although the effect of “free-living” at altitude may reflect a more realistic situation for the traveller at altitude a controlled diet may also have been of benefit in order to draw conclusions with regards to the effect of energy balance through energy intake and its subsequent effects on substrate use, and subsequent alterations in body mass. Also in experimental study 4 (Chapter 7) a lack of physical activity data may be considered a limitation as activity will also effect daily EE during the sojourn and utilisation of fuel throughout the day.
- The recruitment and testing of both males and females is a potential source of increased variation in results, particularly with regard to measures of resting metabolic rate, EE and substrate utilisation. Furthermore a lack of control of the menstrual cycle and/or contraceptive medication in females is a limitation to this thesis and again is a likely source of variation within the results.

- The lack of venous blood sampling during the altitude stay in experimental study 4 (Chapter 7) resulted in measures of Metrn1 being taken prior and upon return to sea-level only. Therefore, the effect of altitude living on immediate changes in this measure cannot be established from current data and should be considered a limitation to the work.
- Although measures of self-reported appetite were taken throughout the altitude stay during experimental study 4 (Chapter 7), potentially important measures with regards to appetite regulation were not taken including the hormones Leptin, Ghrelin and PYY resulting in difficulty in drawing conclusions with regards to the underlying mechanisms of changes observed.

9.6 DIRECTIONS FOR FUTURE RESEARCH

Both findings and limitations presented from experimental studies in this thesis highlight directions for relevant and future research. A model highlighting the contributing factors of body mass loss upon exposure to an environment of reduced oxygen availability has been proposed within this thesis. Although the presented model is based on the collected data it supplies a broad framework from which intervention strategies can be focused upon. Within this model the relative contribution from each interacting factor is likely to demonstrate variability between individuals therefore future research should firstly seek to replicate the findings of the thesis under conditions of acute and intermediate length exposure to reduced oxygen availability.

This thesis has provided studies to support future research in developing a more comprehensive understanding into the mechanisms responsible for the alterations in body mass upon exposure to hypoxia and/ or altitude. Future research should aim to elucidate further the effect of exposure to acute hypoxia on alterations in metabolic rate and more specifically focus on how these changes, when used repeatedly over a prolonged intervention, contribute to a meaningful difference in body mass. In addition future work should aim to establish further the magnitude of observed alterations in circulating blood plasma FFAs and TAGs following passive exposure to severe hypoxia of differing lengths and when experienced repeatedly over a prolonged intervention. Such work will aid in the understanding of the use of hypoxia in an intervention type strategy on blood lipid responses and further highlight the usefulness of hypoxia and altitude living for improvements in health parameters.

An important area of future research is the effects of differing environmental conditions combined with physical activity on the “browning” of adipose tissue. Brown adipose tissue is a possible mediator in the treatment and prevention of metabolic disorders (Cannon and Nedergaard 2004) due to an observed uptake and combustion of glucose and lipid within the tissue (Bartelt et al., 2011; Van Marken Lichtenbelt et al., 2009), and improved insulin sensitivity (Stanford et al., 2013) and increased EE (Lowell and Spiegelman 2000) observed in line with an increase in BAT. Recent research has examined the effect of cold exposure and exercise on a novel form of the transcriptional coactivator PGC-1 α (PGC-1 α 4) (Ruas et al., 2012). Specifically, mice skeletal muscle-specific transgenic over-

expression of PGC-1 α 4 demonstrate muscle hypertrophy, increased basal EE and increased browning of white fat depots without changes in food intake or movement (Rao et al. 2014; Ruas et al. 2012). Expression of PGC-1 α 4 in skeletal muscle stimulates increased mRNA expression and secretion of the hormone Metrnl which promotes thermogenesis. Acute cold exposure and concurrent exercise has been shown to increase the expression of Metrnl (Rao et al., 2014). Acute severe exposure to hypoxia did not induce changes in circulating plasma Metrnl in the present thesis. Likewise an intermediate length stay at 3,400 m induced no changes in Metrnl when measured upon return to sea-level at one and four weeks.

In line with a current growing area of research however future studies should aim to further examine the use of reduced oxygen availability on Metrnl and on the expression of PGC-1 α 4. Measurement of Metrnl at various stages during a prolonged altitude stay would be worthy of investigation. Furthermore, the investigation in humans of reduced oxygen availability combined with exercise modes that have previously been shown to elicit increased expression of PGC-1 α 4, including downhill running and concurrent resistance and endurance training exercise, would be worthwhile. The effects of differing lengths of exposure and severities of altitude on the browning of white adipose tissue should be examined as should the time course of these changes and the time frame in which any changes remain upon return to sea-level as this would be of practical importance for an intervention. As previously mentioned however, it must also be considered that hypoxia may, in fact, have induced a blunting effect on thermogenesis. Although cold exposure has demonstrated strong effects on brown adipose tissue thermogenesis, hypoxia has been observed to have a dampening effect on the normal rise in thermogenesis observed in the cold (Blatteis & Lutherer 1976; Gautier et al. 1991). This blunting effect is suggested to result from a reduction in non-shivering thermogenesis and reduced BAT activity impaired by the hypoxia induced increase in sympathetic nervous activity (Gautier et al., 1991; Madden and Morrison, 2005). Therefore, findings within the current thesis, may be as a result of an opposing effect of hypoxia on Meteorin-like.

The present thesis used recreationally active, normal weighted participants throughout and thus, findings from the thesis are limited to this population. Although the thesis was a mechanistic based body of studies, future work should focus on the use of acute exposure to hypoxia in a group of metabolically unhealthy, overweight participants in order to assess the response of the intervention on such groups, where weight management and loss of adiposity may be beneficial for health. Alterations in metabolic rate and substrate use in an obese population following exposure to hypoxia should be investigated. These effects should further be examined in individuals with a range of body mass' and BMI's in order to better understand any "threshold" of body mass at which the use of altitude is more or less suitable and effective.

Changes in appetite and eating habits upon prolonged exposure to altitude have previously been reported at high altitude (5,000 m) (Westerterp-Plantenga 1999). The release of adipose tissue

hormones such as Leptin and Ghrelin may mediate reductions in feeding (Smith et al., 2011). Measurement of these and other appetite regulating hormones including PYY should occur in conjunction with future work on the sensation of taste when individuals are exposed to an environment of reduced oxygen availability. Future work should also aim to establish both the acute and the more prolonged effects of hypoxia on taste and its correlation with altitude and subsequent eating during a controlled buffet meal. These studies would increase our understanding on the interplay between the anorexic effects of altitude and the contribution to those effects of taste which may subsequently inform future intervention strategies.

Throughout experimental study 4 (Chapter 7) individuals were not instructed with regards to the amount of physical activity to conduct, nor were they constrained in this manner. As such, there was most likely variation in the daily and physical activity energy expenditures of the participants. Previous studies have established beneficial findings when regular exercise training programmes are conducted during exposure to normobaric hypoxia in the chamber setting (Haufe et al., 2008; Netzer et al, 2008; Wiesner et al, 2010). Future research should aim to establish the effects of a more prolonged, continuous stay at true, hypobaric altitude in combination with an individualised and structured training programme on the outcomes of body mass and metabolic parameters of health. Furthermore, differing intensities and modes of exercise should be investigated in this environment in order to establish any differences in metabolic outcomes at altitude between type, frequency and intensity of activity. Energy balance at altitude has been shown to contribute to alterations in substrate use and changes in body mass (Brooks, 2014). In a similar vein, future studies may also benefit from establishing the use of hypoxia and altitude living in conjunction with dietary interventions of differing severities and compositions in comparison to the use of the same constraint of energy intake at sea-level. Moreover, these dietary interventions may also be viewed in conjunction with the previously mentioned training programmes in order to establish the effects of a multi-factorial lifestyle intervention for body mass and metabolic health parameters at altitude compared to sea-level.

Taken together, future work should focus on elucidating the underlying mechanisms and signalling pathways of reduced oxygen availability on the effect of alterations in body mass and cardio-metabolic health measures. In a practical sense, this will allow for the feasibility of a safe and effective intervention strategy incorporating hypoxia and exercise to be developed.

9.7 CONCLUSION

The overriding aims of the current thesis were to examine the contributing factors to changes in body mass upon exposure to an environment of reduced oxygen availability. A multifactorial approach was incorporated within the thesis with the investigation of acute, severe exposure alone and with the addition of moderate physical activity. Furthermore residency for 18 days at an altitude of 3,400 m in free living conditions was also incorporated in order to compare differing lengths and modes of

exposure. Measures of resting metabolic rate, substrate use, appetite, blood lipids and body mass form the bases of the conclusions drawn within this thesis. The effect of acute and intermediate length exposure to hypoxia/altitude on plasma MetrnI concentration adds novelty to the thesis. The combination of both laboratory based acute studies and a longer term study during a period of altitude living is also noteworthy. Results from this thesis further support both the potential use of hypoxia as a useful tool to induce beneficial metabolic adaptations in unhealthy populations, and the cachexic effect of altitude. Results from work conducted in this thesis suggest that increased metabolic rate and reduced energy intake due to a reduced drive to eat, possibly mediated by changes in taste, are the main contributors to loss of body mass during prolonged stay at altitude. Acute interventions of severe exposure to hypoxia are sufficient in altering substrate use and metabolic rate yet further work is required in order to determine if these changes are potentially useful for reducing body mass in humans. Future work should therefore focus on optimising methods of exposure and physical activity in an attempt to fully harness the effect of both interventions for loss of body mass. Further work is also required in order to further elucidate the underpinning physiological mechanisms resulting in metabolic adaptations at altitude. Finally the effect of reduced oxygen availability on the “browning” of adipose tissue and subsequent alterations in metabolic markers including insulin sensitivity, EE and glucose tolerance should take priority.

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APPENDICES

PART 1 - INFORMED CONSENT

DECLARATION

I..... hereby volunteer to take part in Ben Duncan’s study during the period between / / 201.. to / / 201..

My replies to the above questions are correct to the best of my knowledge. I understand that any information collected, including results of any testing will be treated with the strictest confidence. I have been fully informed of the potential risks/discomforts associated with the testing, and I understand the purposes of the study.

I understand that I may withdraw from participating at any time during the testing period, and I am under no obligation to give any reason for this withdrawal.

I agree to obey the laboratory rules/regulations and instructions regarding safety, subject only to my right to withdraw declared above.

Signature of subject:
/ 2014

Date: /

Signature of experimenter:
2014

Date: / /



University of Brighton

PLEASE READ THE FOLLOWING CAREFULLY

You will be considered unfit to take part in this experiment if you:

Have a fever; suffer from fainting spells or dizziness

Have spent prolonged periods at a high altitude within the last two months

Have a known history of medical disorders i.e. high blood pressure, heart or lung disease

This study involves exposure to hypoxic conditions which carries some potential risks and/or discomfort. All testing, however has been approved by an ethics committee and will follow strict health and safety guidelines. You are free to withdraw from the study at any time without justification.



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