NUTRITION AND METABOLIC ADAPTATION: The Assessment and Impact of Dietary Manipulation on Metabolic and Cellular Perturbation.

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A thesis submitted to the University of Hertfordshire in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Declaration

I declare that this work has not previously been accepted in any substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: ……. (Candidate)

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Synopsis

It is well established that improved nutritional strategies can enhance both health and exercise performance. Scientific developments in recent years have furthered our understanding of cellular metabolism, which in turn, has provided an additional platform to investigate the impact of diet on health and adaptation. The overall aim of this research programme was to build on the current understanding of dietary intake in athletes and the impact dietary manipulation has on cellular and metabolic adaptation at rest and in combination with endurance training.

It is postulated that nutrition is the most controllable risk factor impacting long-term health and chronic disease (World-Health-Organization, 2003), and enhanced knowledge of nutrition has been associated with improved dietary choices. A number of nutrition knowledge questionnaires have been developed to assess this; however the validity of each tool is reduced if implemented outside the target population. A valid and reliable general and sport nutrition knowledge questionnaire had not yet been developed. Using a parallel groups repeated measures study design (N = 101) the aim of the first experimental Chapter (Chapter 4) was to develop a new tool to measure general and sport nutrition knowledge in UK track and field athletes. Following the questionnaire design 53 nutrition educated and 48 non-nutrition educated participants completed the questionnaire on two occasions separated by three weeks. The results of the process demonstrated face and construct validity from the development of the question pool, content validity (the nutrition educated group scored > 30% higher that the non-nutrition educated group), reliability (test - retest correlation of 0.98, $p < 0.05$) and internal consistency (Chronbach's alpha value > 0.7) as such establishing a new tool (Nutrition knowledge Questionnaire for Athletes (NKQA)) for the assessment of general and sport nutrition knowledge in track and field athletes.

Athletes' diets are commonly reported as inadequate and previous work has demonstrated a weak positive relationship between diet quality and nutrition knowledge. Additionally a commercially available tool, the metabolic typing questionnaire, claims to identify individual metabolic function and subsequently prescribe a personalised diet to optimise health. Thus the aim of the second

experimental Chapter (Chapter 5) was to quantify nutrition knowledge (using the questionnaire developed in Chapter 4), measure diet intake and quality and investigate the efficacy of the metabolic typing questionnaire in UK track and field athletes. Using a parallel groups repeated measures design participants (UK track and field athletes n = 59, and non-athletic control group n = 29) completed a food diary, the NKQA and the metabolic typing questionnaire at two time points through the year (October and April) to investigate seasonal change. The results of the metabolic typing questionnaire concluded that 94.3% of the participants were the same dietary type and would subsequently have been prescribed the same diet. Athletes possess greater general and sport nutrition knowledge the non-athletes (60.4 ± 2.0 % vs. 48.6 ± 1.5 %) and also had better diet quality (76.8 ± 10.5 % vs. 67.6 ± 2.6 %). However no relationship was observed between individual nutrition knowledge score and diet quality (r^2 = 0.003, p = 0.63). No difference in dietary intake was observed between power and endurance athletes; average diet intake consisted of 57.0% carbohydrate, 17.1% protein and 25.9% fat.

The metabolic typing diet is based around three different diets: high carbohydrate, high protein and mixed diet. The results from Chapter 5 identified that the metabolic typing questionnaire was not able to differentiate between metabolic function in healthy individuals. Additionally all athletes, independent of event (power vs. endurance), consumed similar diets. With such similarities a clearer understanding of the impact such diets have at a cellular level is required. Therefore for the remainder of the thesis it was decided to investigate the impact of dietary manipulation utilising more robust measures.

Mitochondria are responsible for energy production; their quantity and density have been associated with improved health and endurance performance. External stressors such as energy reduction, carbohydrate restriction and exercise are potent stimulators of transcription markers of mitochondrial biogenesis. Thus manipulating carbohydrate and energy availability *in vivo* may enhance cellular adaptation and limited literature exists on the impact increased protein intake has on this. The aim of Chapter 6 was to investigate the impact of acute (7-day) continuous dietary manipulation on metabolic markers, body composition and resting metabolic rate (RMR). Using a repeated measures parallel group (N = 45) design, participants were randomly assigned one of

four diets: high protein hypocaloric, high carbohydrate hypocaloric, high protein eucaloric or high carbohydrate eucaloric. The macronutrient ratio of the high protein diets was 40% protein, 30 % carbohydrate and 30% fat, the high carbohydrate diets were 10% protein, 60% carbohydrate and 30% fat. Energy intake in the hypocaloric diets was matched to resting metabolic rate (RMR). Participants consumed habitual diet for 7-days then baseline measures were collected (skeletal muscle biopsy, dual energy X-ray absorptiometry scan (DXA) and RMR, habitual diet was consumed for a further 7-days and repeat testing was completed (these time points were used as a control), the intervention diet was then consumed for 7-days and post measures were collected.

The results of the skeletal muscle biopsy demonstrated no group x time interaction in any marker, however a pre-post time difference subsequent to the high protein hypocaloric diet (the diet which induced the greatest metabolic stress) was observed in four transcriptional markers of mitochondrial biogenesis (pre-post intervention fold increase: PCG1-α 1.27, AMPK 2.09, SIRT1 1.5, SIRT3 1.19, $p < 0.05$). The results of the DXA scan demonstrated that the high protein hypocaloric group lost significantly more fat mass than the high carbohydrate eucaloric group (-0.99 kg vs. -0.50 kg, $p < 0.015$). Irrespective of macronutrient ratio, no energy-matched between group difference was observed in lean mass (LM) loss. However when matched for macronutrient ratio the high protein diet attenuated LM loss to a greater extent that the high carbohydrate diet, suggesting an important role of increased protein intake in the maintenance of lean mass. No time point or group difference in RMR was observed. This data suggests that a high protein low carbohydrate hypocaloric diet may provide a stimulus to promote skeletal muscle metabolic adaptation.

The aim of the final experimental Chapter (Chapter 7) in this thesis was to explore the impact exercise in combination with a high protein diet on metabolic adaptation, substrate utilisation and exercise performance in well trained runners. Using a parallel groups repeated measures study design the participants (well-trained endurance runners, N = 16) consumed normal habitual diet for 7-days, then 7-days intervention diet (high protein eucaloric or high carbohydrate eucaloric, same dietary ratios as Chapter 6) and finally returned to habitual diet for 7-days, training was consistent throughout. A pre exercise muscle biopsy was taken subsequent to each diet and

immediately followed by a 10 km sub-maximal run and a time to exhaustion run (TTE) at 95% of velocity at maximal aerobic capacity ($v\dot{V}O_2max$). Post intervention the high protein group presented significant changes in sub-maximal substrate utilisation with 101% increase in fat oxidation (0.59 g•min^{-1}, p = 0.0001). No changes were observed in substrate utilisation in the high carbohydrate group. A trend towards a reduction in average weekly running speed was observed in the PRO group (-0.9 km•h^{-1}), the high carbohydrate group maintained the same training speed. TTE was decreased (-23.3%, p = 0.0003) in the high protein group subsequent to the intervention, no change was observed in subsequent to the high carbohydrate diet. The high carbohydrate group demonstrated preferential increases in markers of metabolic adaptations (fold increase: AMPK = 1.44 and PPAR = 1.32, $p < 0.05$) suggesting that training intensity, rather than carbohydrate restriction, may be a more profound driver of metabolic adaptation. All performance measures, in both groups, returned to pre intervention levels once habitual diet was returned; however the increased gene expression observed in the high carbohydrate group remained elevated 7-days post intervention. The increased metabolic stress imposed by reducing carbohydrate intake did not increase transcriptional markers of mitochondrial biogenesis. For continuous endurance training and high intensity endurance performance a high carbohydrate diet is preferential to a high protein diet.

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I will definitely not be the first (or last) doctoral student to say the process was tough, not just on me but everyone I know! Doing my Ph.D has been part of my identity over the last 5-6 years. I am proud of my research, my thesis and my development throughout this time.

I am so grateful for my girlfriend → fiancée → wife who has been with me throughout the whole journey. Maxine Furber – you are my world. Your support, understanding and love have been integral to my thesis completion; I look forward to having our weekends back and raising our first child. Thank you for everything you have done – I owe you a lot! I would also like to thank my family and friends who have lived this journey with me, in particular my Mum, Dad and sister who's love and understanding have been incredible over the years.

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List of Abbreviations

The following abbreviations have been defined in the test in the first instance

ACSM	American College of Sports Medicine
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BM	Body mass
BMI	Body mass index
BMR	Basal metabolic rate
CHO	Carbohydrate
cm	Centimetre
CNS	Central nervous system
CON	Control
Cp	Crossing point
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DQI	Diet quality index
DXA	Dual energy X-ray absorptiometry
EAR	Estimated average requirement
EI	Energy intake
EM	Energy matched
END	Endurance
ER	Energy restricted
ERR	Estrogen-related receptor
FAT	Fat
FFQ	food frequency questionnaire
FM	Fat mass
FOXO1	Forkhead box O1
FXR	Farnesoid X receptor
g	Grams
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GEM	Gas Exchange Measurement
GEN	General nutrition
GPS	Global positioning satellite
GSK	GlaxoSmithKline
h^{-1}	Hour
HC	High carbohydrate
HF	High fat
HNF	Hepatic nuclear factor
HP	High protein

HTA	Human tissue act
HYD	Hydration
kcal	Kilogram calorie
kg	Kilogram
km	Kilometer
KO	Knock out
LC	Low carbohydrate
LF	Low fat
LG	Low glycogen
LKB1	Liver kinase B1
LM	Lean mass
LP	Low protein
LT	Lactate threshold
MaxSE	Maximal sustainable effort
min	Minute
mL	Millilitre
mm	Millimetre
mmol	Milimoles
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
mRNA	Messenger ribonucleic acid
MT	Metabolic type
MTQ	Metabolic typing questionnaire
N	Number of participants
NAD	Nicotinamide adenine dinucleotide
NG	Normal glycogen
NKQ	Nutrition knowledge questionnaire
NKQA	Nutrition knowledge questionnaire for athletes
NONUT	No nutrition trained group
NRF	Nuclear respiratory factor
NUT	Nutrition trained group
^{o}C	Degrees Celsius
PAF	Physical activity factor
OS	Oxidative system
PCr	Phosphocreatine
PGC-1α	Proliferator persoxisome-acitvated receptor coactivator-1 alpha
POW	Power
PPAR	Peroxisome proliferator-activated receptors
PRO	Protein
qPCR	Quantitative polymerase chain reaction
RDA	Recommended daily allowance
RMR	Resting metabolic rate
RNA	Ribonucleic acid
RNI	Reference nutrient intake

RPE	Rating of perceived exertion
RPM	Revolutions per minute
SD	Standard deviation
SEM	Standard error of mean
SIRT1	Silent information regulator T1
SOP	Standard operating procedure
SPO	Sport nutrition
SPSS	Statistical Package for the Social Sciences
SS	Steady state
TEA	Thermic effect of activity
TEE	Total energy expenditure
TEF	Thermic effect of food
TP	Time point
TR	Thyroid receptor
TTE	Time to exhaustion
UCP1	Uncoupling protein-1
UK	United Kingdom
uL	Microliter
$\dot{V}CO_2$	Volume of carbon dioxide
$\dot{V}O_2$	Volume of oxygen
$\dot{V}O_2max$	Maximal oxygen consumption
WT	Wild type

Collaborations

I would like to thank Charlotte Ashby and Emma Koppe, both GSK employees in the RD Platform Technology & Science department. Their support, training and guidance on the qPCR analysis of the muscle samples was invaluable.

Table of collaborations and contributions.

Chapter	**Collaborator**	**Contribution**
Chapter 6	Charlotte Ashby (CA) and Emma Koppe (EK).	Alongside MF, EK and CA helped develop the qPCR methodology and supported with the isolation, reverse transcription, quantification and amplification of the extracted mRNA from the muscle tissue (MF, CA and EK were present throughout all extraction processes. All equally contributed; ~33% of the process each)
	Ana Antón-Solanas (AAS).	MF developed the DXA scanning protocol. AAS completed ~75% of the DXA scans in this Chapter. The remaining 25% were completed by MF.
Chapter 7	Charlotte Ashby (CA).	Alongside MF, CA supported the isolation, reverse transcription, quantification and amplification of the extracted mRNA from the muscle tissue (MF and CA were present throughout all the extraction processes and contributed equally; ~50% to the process each)

Preface

Some of the results presented in this thesis have been published in the following communications:

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Furber, M., Anton-Solanas, A., Koppe, E., Ashby, C., Roberts, M., & Roberts, J. A 7-day high protein hypocaloric diet promotes cellular metabolic adaptations and attenuates lean mass loss in healthy males. *Clinical Nutrition Experimental*. doi:10.1016/j.yclnex.2017.05.002

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GSK Human Performance Lab – November 2015
Symposium: Nutrition for Endurance Athletes
The use of Metabolic Typing as a tool for Dietary Individualisation

University of Hertfordshire Annual Life Science Conference – April 2014
Oral Presentation:
Nutrition Knowledge and Metabolic Type in UK Track and Field Athletes.

1 PRELUDE

The relationship between dietary intake and exercise performance has long been the subject of research. It is now well established that nutritional intake can impact both health and exercise performance and different exercise modalities may require specific fuelling strategies (Thomas et al., 2016). With developments in research the concept of personalised nutrition has rapidly progressed (Joost et al., 2007), consequently a number of theories have been derived which claim to provide an individual approach to dietary prescription. One such method is the metabolic typing questionnaire, which, through a series of questions, claims to differentiate dietary requirement and subsequently prescribe individual nutrition programmes (Walcott and Fahey, 2000). With regards to the athletic population, despite this greater understanding of dietary requirements, athletes' diets are commonly reported as inadequate. The reason for this is still unclear (Heaney et al., 2011), however it is possibly related to the differing physiological demands of multiple sports and the varied fuelling strategies required to fuel the metabolic processes (Phillips, 2006).

Mitochondria are responsible for energy production, mitochondrial quantity and density have been linked with increased resting metabolic rate (Larsen et al., 2011) and endurance performance (Psilander et al., 2013). The transcription coactivator proliferator persoxisome-acitvated receptor coactivator-1 alpha (PGC-1α) has been highlighted as a primary regulator of mitochondrial biogenesis which, in turn, is activated by AMP-activated protein kinase (AMPK) and Silent information regulator T1 (SIRT1) both of which are stimulated by environmental stressors such as dietary manipulation and endurance exercise.

Given the high training demands of the modern day athlete the ability to optimise training adaptation from each session has become paramount. Increasing metabolic stress during endurance exercise, by restricting carbohydrate availability, has been demonstrated to up-regulate the cellular response and enhance exercise efficiency (Hawley and Morton, 2014). However, the majority of research has demonstrated no change or even a decrease in endurance performance when accompanied with carbohydrate restriction (Burke, 2015). Generally, increasing dietary fat intake attains

carbohydrate restriction. High protein, low carbohydrate diets are currently *en vogue* and the impact of such diets, independently, and in combination with endurance training, on skeletal muscle adaptation and endurance performance *in vivo* are yet to be fully explored.

The literature review is comprised of two distinct parts. PART 1 introduces the theory of metabolic typing and the underlying principles of its application, a brief overview on the metabolic demands of speed and endurance exercise, and the scientific principles of nutrition knowledge assessment. PART 2 is a critical analysis of the metabolic pathways that control mitochondrial biogenesis, with particular reference to external stressors such as dietary manipulation and exercise. Additionally PART 2 provides review of the current literature surrounding the manipulation of dietary intake and the impact this has on subsequent exercise performance. The aims of this series of investigations are: (i) to assess nutrition knowledge and dietary habits of UK track and field athletes, and (ii) to assess the efficacy of the metabolic typing questionnaire as a tool to differentiate dietary individuality. The second phase of research is to provide further insight into the impact of high protein diets on cellular and metabolic adaptation at rest and accompanied with endurance exercise.

2 LITERATURE REVIEW

2.1 PART 1

2.1.1 The theory and application of the metabolic typing diet.

The method of prescribing population specific nutritional recommendations is not new – for example, guidelines for infants, diabetics and pregnant women have existed for decades, however with enhancements in genomic and metabolic research the concept of personalised nutrition has rapidly progressed (Joost et al., 2007). More recent advances have opened up the possibility for nutrition to be personalised to an individual's characteristics and not just group classification (Joost et al., 2007). The metabolic typing diet (Walcott and Fahey, 2000) is a commercially available method which utilises a self-assessment questionnaire to prescribe a diet suitable to match metabolic type; claiming to be a personalised approach to nutrition. The questionnaire is used by practitioners and consumers alike, however to date little scientific evidence exists examining its application and effectiveness.

Individuality pervades every aspect of the human body, the internal anatomical characteristics of humans are every bit as variable as the external characteristics (Williams, 1956). These inherited differences extend to the structure and metabolism of every cell and determine the speed and efficiency with which cells perform their essential functions (Wolcott & Fahey 2000). The basis of this statement were built on the foundations of Williams (1956) original work on biochemical individuality which indicated that the variations of metabolic biochemistry are almost infinite. Adopting the commonly accepted demarcation between being normal and abnormal as 0.95, it would be assumed that 95% of the population is normal in respect to one measurable item. However with two uncorrelated measurable items 90.2% (0.95^2) would be classified as normal, and with 10 and 100 (95^{10} and 95^{100}) uncorrelated items 0.6% and 0.059% would be classified as normal, thus producing our very own 'individual self'. *Homo sapiens* are seen to be homogenous, however this highlights that we are all fundamentally disparate (Williams, 1956). Such structural disparity could potentially lead to differences in metabolic function.

The fundamental principles of the metabolic typing diet are that biochemically disparity exists within everyone, and two dominant regulators of human metabolism control metabolic output: the oxidative system (OS) (the speed at which food is converted into energy) and autonomic nervous system (ANS) (neurological control of metabolism via the ANS and enteric nervous system) (Wolcott and Fahey 2000). Each system is then subdivided into two further branches: fast oxidisers and slower oxidisers within the OS; sympathetic and parasympathetic control within the ANS. Kristal and Haig (2002) state that in everyone there lies a metabolic dominance within one of these systems, and that these primary controls of metabolism (metabolic type) can have a predetermined effect on metabolic function, requiring different nutrition strategies to optimise metabolism.

In the preface of Kristal and Haig (2002) book titled 'The Nutrition Solution: A Guide to Your Metabolic Type', the opening line states: "Most health-conscious people are aware of the necessity to eat good food and take supplements". Similar assumptions within the opening line are synonymous throughout the book. Kristal and Haig (2002) then proceed to highlight numerous cases where the application of a metabolic typing diet has cured chronic health issues where traditional medicine has not been successful. Equally Walcott and Fahey (2000) (book entitled 'The Metabolic Typing Diet') highlight numerous cases of chronic illness being cured utilising the metabolic typing diet. Superficially the method and application are logical, however with no research on the application and a number of bold statements throughout the books, caution should be applied if utilising the technique and further research is needed.

2.1.2 A critical analysis of the metabolic typing diet.

It is well established that changes in diet composition can produce large, rapid shifts in oxidation rates and basal metabolic rate (Folch et al., 2001). It has also been shown that when total energy is fixed and diet composition is altered, the body rapidly changes oxidation rate in response to changes in intake (Burke et al., 2002). Carbohydrate oxidation has also been shown to reduce with a decreased carbohydrate intake and elevate with an increased intake (Peters and Leblanc, 2004). The manipulation of diet to suit the energy needs of the individual could provide health

and performance benefits (Burke, Millet & Tarnopolsky, 2006). However large inter-individual responses to dietary manipulation have been observed, supporting the theory that ‘not one diet fits all’ (Wolcott & Fahey 2000). Therefore it is important to consider each individual as an individual, opening up the question whether metabolic typing (i.e. matching dietary intake to individual needs) is able to provide this.

The ‘metabolic typing diet’ is based around the theory of homeostasis and the importance of maintaining a stable equilibrium between independent physiological variables. The theory states that imbalances within eight homeostatic controls (i) the oxidative system (intracellular energy conversion); (ii) ANS (master regulator); (iii) catabolic/anabolic system (oxidative metabolism); (iv) endocrine type (food selection and weight control); (v) acid/alkaline balance (pH); (vi) prostaglandin balance (inflammatory responses); (vii) constitutional type (link between food and metabolism) and (viii) electrolyte balance (circulation and osmotic pressure) (Walcott and Fahey, 2000) can be corrected by the application of the metabolic typing diet.

Literature exists supporting both the impact dietary intake has on each of these homeostatic controls and the subsequent impact this has on metabolism. It is well understood that mitochondrial quantity and density can impact metabolic rate and energy conversion, and reduced mitochondrial numbers are linked with diabetes (Su et al., 2013). Furthermore the protein PGC-1α has been demonstrated to play a primary role in the regulation of oxidative phosphorylation (Oxidative system) which is impacted by nutrient availability (Lin et al., 2005). In a recent review article published in Nature, Myers and Olson (2012) suggest that the CNS can, in some cases, dominate the control of metabolic homeostasis by controlling the overall peripheral response (ANS). The maintenance of muscle mass is achieved through a fine balance between muscle protein synthesis and muscle protein breakdown. Nutrient deficiency can results in a state of negative net muscle protein balance, resulting in catabolism (Phillips and Van Loon, 2011) (catabolic/anabolic system). The control of appetite and hunger are influenced by a number of circulating hormones; a disruption in hormone secretion can lead to weight gain. Hormones such as insulin, glucagon, leptin and ghrelin link the control of cellular energy metabolism to the central nervous system by modulating short-term signals that determine meal initiation and termination as well as energy balance (endocrine type) (Mastorakos and Zapanti, 2004). Chronic metabolic

acidosis is associated with severe growth retardation in humans (Nash et al., 1972) and it has been demonstrated to substantially decrease albumin synthesis, contributing towards sustained negative nitrogen balance (acid/alkaline balance) (Ballmer et al., 1995). The metabolic pathway of the immune cell relies heavily on oxidative phosphorylation, which can be impacted by glucose and lipid availability (Ganeshan and Chawla, 2014), and prostaglandins have been demonstrated to both sustain homeostatic function and support the inflammatory response (prostaglandin balance) (Ricciotti and FitzGerald, 2011). Finally it is well established that the quality and quantity of food consumed impacts both the metabolic response and the control of metabolism (Draznin et al., 2012) (constitutional type). As such, if a diet is able to control or restore homeostatic balance in each of these systems, the body would be in balance and a nutrition solution could optimise metabolism.

The metabolic typing questionnaire (Appendix One) is a commercially used method to prescribe individualised dietary intake. Rather than directly measuring and analysing each of these 'homeostatic controls', the metabolic typing diet uses a simple 65 item questionnaire, with each item designed to represent an aspect or function/control of one of the homeostatic mechanisms. Each item has up to three separate answers, with choices classified as either A, B or C. The total number of each answer is calculated and MT is then selected using the following criteria:

- If the number of A answers was 5 or more higher than both B and C, then the individual is classified as a '*Carbohydrate Type*'.
- If the number of C answers was 5 or more higher than both A and B, then the individual is classified as a '*Protein Type*'.
- If the number of B answers was 5 or more higher than both A and C, then the individual is classified as a '*Mixed Type*'.

Once completed, a diet regime and series of supplements are prescribed to match the identified metabolic type. However, fundamentally the prescribed diets are either a high protein, low carbohydrate diet (40–45% protein, 35–40 % carbohydrate); a high carbohydrate, low protein diet (70% carbohydrate, 10% protein); or a mixed diet (50-55% carbohydrate, 30% fat, 15-20% protein) (Appendix One), and therefore not quite as personalised as originally stated.

Within the questionnaire it is important to consider the rational for the selection of each item. Some of the questions in the Walcott and Fahey (2000) metabolic typing

questionnaire are ambiguous and the justification for their presence is not obvious. For example, question 6 in the metabolic typing questionnaire is:

Some people commonly experience chest pressure, a distinct sensation of pressure in the chest area. It often makes people feel as though a weight is on their chest, and tends to inhibit the ability to breathe. *(Only answer if you suffer from chest pressure)*

a.

b.

c. I have a tendency to get or have problems with chest pressure

According to this question only 'protein types' experience chest pressure. Without a justification for the inclusion of this question, it may be presumed that the question alludes to a link between chest pressure and metabolic acidosis. It is well established that when bicarbonate levels in arterial blood are low, pH decreases, resulting in acidosis and hyperventilation which may cause the sensation of chest pressure (Delguercio et al., 1965). Walcott and Fahey (2000) discuss the importance of balancing the blood pH to maintain metabolic equilibrium, stating that a high protein diet (type C) increases acidosis. However a number of other factors could lead to common chest pressure, an excellent paper from Jerlock et al. (2005) discuss the implication of living with unexplained chest pressure, which cannot be attributed to acidosis. A further example of an ambiguous question within the metabolic typing questionnaire is on coughing:

Usually we think of coughing as something associated with illness. But some people naturally cough easily and often even when they are not sick. Typically the cough will be a dry cough and short in duration. If you are one of these people please highlight the answer. *(Only answer if you suffer from regular coughing)*

a. I tend to cough every day

b.

c.

Once again, as with chest pressure, coughing is only associated with one particular metabolic/dietary type. This particular question presumes that carbohydrate types witness symptoms of unexplained coughing, where mixed or protein types do not. An excellent paper from Sifrim et al. (2005) found that chronic unexplained coughing was

clearly associated with weak acid gastro-oesophageal reflux. Thus a weak ability to tolerate acid, not alkaline as the question insinuates. Numerous other question examples exist which are hard to justify their inclusion. However some questions within the questionnaire are supported with more robust science.

"What foods worsen your ability to concentrate?

- **a.** Meat and/or fatty foods.
- **b.** No particular kind of food seems to disrupt my concentration.
- **c.** Fruit and vegetables and grain-based carbohydrates."

It is well established that a meal low in fat and protein can impact blood glucose and insulin levels, subsequently affecting alertness and concentration (Holt et al., 1999). Thus, if a slow oxidiser performs best on a high fat and protein diet then a meal such as fruit and/or simple carbohydrates may be detrimental to concentration and focus.

2.1.3 Summary of the metabolic typing diet and its application.

Measuring biomarkers and prescribing an intervention to enhance that particular aspect of health has been used for decades (Grundy and Denke, 1990). Personalised nutrition is becoming ever popular, and prescribing a diet individualised to a person's metabolic requirements may enhance health and reduce the chance of illness. The metabolic typing diet claims to be a personalised approach to nutrition, and dependent on the outcome of a 65 item questionnaire, will prescribe a diet to reset the bodies homeostatic control, providing a nutrition solution to optimal health. The science supporting the inclusion of some of the questions present within the questionnaire is ambiguous. Numerous case studies have been reported demonstrating rapid improvements in health and metabolic status utilising the metabolic typing diet, however to date, no research exists on the effectiveness of the intervention, both as a tool for individualising dietary intake and its subsequent use to improve health. Furthermore it may be argued that the scientific robustness of the questionnaire is lacking.

2.1.4 Carbohydrate and Protein requirements for speed and endurance athletes.

Energy balance is defined by two components: energy intake and energy expenditure. Energy balance occurs when total energy intake (EI) is equal to total energy expenditure (TEE). TEE in turn consists of the summation of basal metabolic rate (BMR), the thermic effect of food (TEF) and the thermic effect of activity (TEA).

$$TEE = BMR + TEF + TEA$$

TEA = Planned exercise expenditure + spontaneous physical activity + non exercise activity thermogenesis (Thomas et al., 2016)

Chronic perturbations in the two components can lead to fluctuations in body weight. Managing nutritional intake to meet the energy demands of athletic training is key to support adaptation and performance improvements (Thomas et al., 2016). The individual events included within Track and Field cover the full range of the physiological phenotypic characteristics (Figure 2.1.1). For events such as 100 metre sprinting and marathon running a phenotypic dominance at each end of the continuum is essential to perform at the highest level (Joyner et al., 2011, Trappe et al., 2015). A large disparity in characteristics and physiological determinates of performance exist at both ends. Elite power athletes are able to sustain extremely high energy outputs (>20 kcal•min^{-1}) at an intensity greater that 100% $\dot{V}O_2$max, with blood lactate levels in excess of 20 mmol•L^{-1} for short periods of time (Stellingwerff et al., 2011). Whereas endurance athletes can sustain a high aerobic output and levels >90% $\dot{V}O_2$max for prolonged periods (Jeukendrup, 2011). Naturally, with such disparity in the physiological characteristics of these athletes, recommendations for optimal energy and nutritional intake vastly differ.

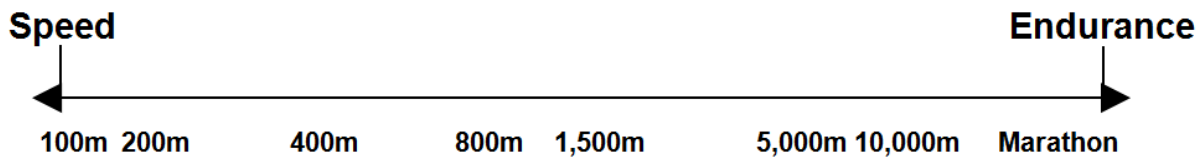


Figure 2.1.1. Physiological phenotypic continuum of the metabolic demands for track and field events

Energy expenditure is very high for endurance athletes due to the volume and intensity of training. It is well established that muscle glycogen is a rate limiter to endurance performance (Bergstrom et al., 1967) and increased muscle glycogen stores pre exercise can prolong subsequent endurance performance. As such carbohydrate intake recommendations for endurance athletes are relatively high, ranging from 6-12 $g \cdot kg^{-1} \cdot d^{-1}$ (50–75% total energy intake) (American Dietetic et al., 2009).

For speed and power events, muscular strength and force development are key performance indicators. Energy expenditure through physical activity is generally lower than endurance athletes, however due to increased body mass and muscle mass overall energy intake needs to be high to match energy expenditure. Due to the supra-maximal and anaerobic nature of the training, carbohydrate requirements are lower than endurance athletes, however given that multiple sprints are common to a sprint training session depletion of muscle glycogen is possible (Gaitanos et al., 1993). Sprinters should consume sufficient carbohydrate to maintain glycogen during training; as such daily carbohydrate intake recommendations of $5g \cdot kg^{-1} \cdot d^{-1}$ (40–50% of daily energy intake) are generally sufficient for sprinters (American Dietetic et al., 2009, Tipton et al., 2007).

The maintenance of muscle mass is a balance between muscle protein breakdown (MPB) and muscle protein synthesis (MPS) (Phillips and Van Loon, 2011). During exercise an increase in protein turnover occurs which increases the daily demand for protein intake. The current UK Reference Nutrient Intake (RNI) for protein, set by the British Nutrition Foundation, is $0.75g \cdot kg^{-1} \cdot d^{-1}$, meeting the needs of ~97.5% of the population. Whereas protein intake guidelines for athletes are designed to optimise the adaptive response to the training stimulus (Thomas et al., 2016), with recommendations ranging from 1.2 – $2.0g \cdot kg^{-1} \cdot d^{-1}$ in athletes (Thomas et al., 2016). It is well established that increased protein intake, and the timing of feeding is key in supporting gains in muscle growth and strength (Phillips and Van Loon, 2011) and individuals with a large muscle mass and higher protein turnover require more protein to remain in a net muscle protein balance (Phillips and Van Loon, 2011). Generally a larger muscle mass is required to perform well in strength and power sports relative to endurance. Additionally, an increased amount of training is focussed on muscular hypertrophy which providing a greater challenge to remain in a net muscle protein balance. As such daily protein recommendations are higher in strength/power athletes;

1.5-2.0 g•kg^{-1}•d^{-1} of protein for strength and power athletes (Tipton et al., 2007) and lower 1.2–1.7g•kg^{-1}•d^{-1} for endurance athletes (Burke et al., 2007).

2.1.5 The use of the metabolic typing diet as a tool for dietary prescription for athletes.

As discussed in Chapter 2.1.2 personalised nutritional programs are still in their infancy. In general, nutritional recommendations for athletes are based on the physiological requirements of the sport rather the nutritional needs of the athlete (Thomas et al., 2016). Due to greater protein requirements for speed/power athletes and increased carbohydrate needs for endurance athletes, along with the distinct physiological differences of power and endurance events; it could be argued that the two groups of athletes have different metabolic needs, thus metabolic type. Endurance athletes maybe classified as metabolic type 'A' (carbohydrate type) and speed/power athletes as metabolic type 'C' (protein types). A comprehensive grasp on the individual metabolic requirements of the athlete may be gained by implementing the metabolic typing questionnaire. The output will prescribe a specific diet to meet the individual's metabolic requirements, not just the physiological requirements of the event. This may lead to improved health and sporting performance. As such the implementation of the metabolic typing questionnaire in athletes may provide clearer recommendations for individual dietary intake; however this is yet to be explored.

2.1.6 Measuring nutrition knowledge in athletes.

For millennia, it has been recognised that nutrition plays an important role in human health. It is postulated that nutrition is the most controllable risk factor impacting long-term health and chronic disease (World-Health-Organization, 2003), which can be easily manipulated to improve exercise performance (Burke and Deakin, 2010). Consequently, optimal health and sport nutrition strategies have been subject to comprehensive research (Magkos and Yannakoulia, 2003). However the sport and fitness industries are saturated with varying opinion, articles and internet material

which can provide unsubstantiated nutrition claims (Smith-Rockwell et al., 2001), as such, recommendations may be controversial and/or misinterpreted. Such contrasting information could appear confusing to those without sufficient exposure to nutrition education.

Athletes' diets are commonly reported as being nutritionally inadequate (Heaney et al., 2011). The underlying reasons for this are unclear, but may include: 1) the athlete knows what to consume but does not do so; 2) the education messages given to the athlete are inaccurate; 3) the athlete is not getting educated in nutrition; 4) the athlete does not think nutrition is an important aspect of performance; and 5) the athlete thinks their current nutrition habits are adequate (Heaney et al., 2011).

Nutrition knowledge, broadly defined, refers to knowledge of concepts and processes related to nutrition and health including knowledge of diet and health, dietary guidelines and recommendations specific to a particular population (Miller and Cassady, 2015). It has been postulated that in order to make healthy food choices it is important to have an internalised and comprehensive structure of nutritional knowledge (Rasanen et al., 2003). A person may master some aspects of nutrition, however if understanding of the connections between different nutritional facts is incomplete the ability to translate this knowledge into decisions impacting good food choice is compromised (Cotugna et al., 1992). To better understand nutritional knowledge a range of nutrition knowledge questionnaires (NKQ) have been developed, each with its own advantages and disadvantages and designed for a specific target population; New Zealand rugby coaches (Zinn et al., 2005), South African adolescents (Whati et al., 2005), elderly (Thomas et al., 1990), inpatients (Anderson et al., 1988); all having limited application when removed from the target population. Furthermore, few of the questionnaires address nutrition knowledge for an athletic population, and none specific to the UK athlete (Table 2.1.2).

Designing a construct valid questionnaire requires a lot of time and a significant attention to detail, thus reasonably it should be resorted to only if an existing instrument cannot be found (Parmenter and Wardle, 1999). As such, many authors may select existing or un-validated tools for ease of data collection; however the questionnaire may not accurately access the research question. A primary advantage for designing

a new measure is that it will, if designed correctly, ensure that the NKQ will directly address the research question.

A test is referred to as valid if it measures what it claims to measure (Kline, 2007a), for a questionnaire to be valid and reliable a series of psychometric measures must be performed. Psychometrics, the science of measuring or scaling psychological attributes, has a defined set of criteria to formulate a valid test. These include tests of validity: content, construct and face, along with tests of reliability: test re-test and internal consistency (Table 2.1.1) (Kline, 2007a). Additionally, when defining the scope of the measure it is important to distinguish between 'knowledge' and 'beliefs'. Knowledge can be conceptualised as factually true and knowledge items can be judged as correct or incorrect, where as beliefs may be more complex than just true or false (Parmenter & Wardle 1999). If a questionnaire is to contain both 'knowledge' and 'belief' questions then it is imperative that the two question groups are dissociated from each other within the results.

The use of a previously validated NKQ can be a very productive tool if selected wisely; Jessri et al. (2010) assessed the nutritional knowledge of Iranian college students using the psychometrically valid and reliable questionnaire developed by Zinn et al. (2005). The questionnaire was previously designed specifically for New Zealand rugby coaches; importantly however the questions were sport specific thus providing enough evidence to draw a strong conclusion. However the use of previously validated questionnaires can be equally ineffective; Pessi and Fayh (2011) evaluated the nutritional knowledge of professional Brazilian track and field athletes and triathlete's.

Table 2.1.1. Steps Taken to Assess for Validity and Reliability of a questionnaire (Adapted from Kline (2007b)).

Measure		Definition	Method	Statistical Analysis
Validity	**Content**	The extent to which a measure represents all facets or the area of interest	Questionnaire developed with expert opinion.	N/A
	Construct	The degree to which the test measures what it claims to be measuring.	Questionnaire administered to two different groups, with different training in subject area. Significant different scores achieved.	Independent samples T-Test
	Face	The extent to which a test is subjectively viewed as covering the concept it purports to measure	Questionnaire appears to be measuring what it claims to measure.	N/A
Reliability	**Test-retest**	The degree to which test results are consistent over time.	The correlation of the scores from a set of subjects who have taken to test on two separate occasions.	Pearson's Correlation
	Internal consistency	A measure of how well the items on the test measure the same construct / idea.	To measure the inter-correlation of the questions within the questionnaire.	Chronbach's alpha

Thirty-seven males and 13 females (age 32.4±11.1 years) completed the 12 item questionnaire (originally developed by Harnack et al. (1997) to assess the nutritional knowledge, beliefs and attitudes of cancer patients). Although some of the questions were relevant others were less so, with questions focused on the relationship between food and cancer and whether food can prevent cancer. These were appropriate for Harnack et al. (1997) however not so for Pessi and Fayh (2011). Seeking knowledge about which foods impact cancer development is not essential knowledge for an

athlete, as in general, an/the athlete primary nutritional concern is performance (not combating chronic ill-health), thus due to the use of this tool caution should be had with any conclusions drawn from the study. Pessi and Fayh (2011) found no significance in their results however concluded that the majority of the athletes presented satisfactory nutrition knowledge. This paper highlights the importance of constructing a strong methodological design (Kline, 2007b). If the questionnaire addressed various nutritional issues relevant to track and field athletes, the findings may expose a specific gap in the athlete's knowledge, thus offering an education pathway for the user.

To the author's knowledge, in the last 15 years only three studies have investigated the nutrition knowledge of athletes relative to a non-athletic control (Table 2.1.2) (Raymond-Barker et al., 2007, Cupisti et al., 2002, Spendlove et al., 2012). In a population of young Italian females (athletes: n = 60, non-athletes n = 59, age = 14 to 18 years) spanning a range of sports, Cupisti et al. (2002) demonstrated that athletes produced a significantly higher rate of correct answers on the nutrition knowledge questionnaire relative to non-athletes (77.6 % vs. 71.6 %, $p < 0.01$). Although statistically significant, the 6 % difference in NKQ score observed is relatively small. The NKQ used was assessed for internal consistency, however the steps taken to ensure construct validity were not present raising a question over the complexity of the research tool. The high rate of correct answers (77.6%) is the second highest reported from an athletic population and is almost as high as the mean correct response of dietetic trained participants (86.2%; reported by Spendlove et al. (2012)). Spendlove et al. (2012) demonstrated no significant difference in nutrition knowledge between the elite Australian track and field athletes (57.6%) and non-athlete group (63.1%), with the non-athlete group performing slightly better.

Both Raymond-Barker et al. (2007) and Spendlove et al. (2012) used the validated general nutrition knowledge questionnaire developed by Parmenter and Wardle (1999), however it was not specific to an athletic population. Due to the absence of a sports nutrition section caution should be taken when extrapolating these results and drawing conclusions on the nutrition knowledge of an athlete.

Table 2.1.2. Nutrition knowledge of athletes versus non-athlete comparison groups

(Adapted from Heaney et al. (2011)).

	Athletes (A)			Comparison Group (C)			Questionnaire Summary			Knowledge Scores	
Reference	*N* (gender)	Sport, level	Age (years)	*N* (gender)	Description	Age (years)	Tool design	No. of items, question type	Validation	Scores (%)[a]	Sig
Barr, 1987	70 (F)	Mixed, tertiary	NR	129 (F)	University students psychology major	NR	SDQ	87 T/F GN, SS	PT, CV-diet	A 34, C 34	A = C
Collison et al., 1996	28 (F)	Mixed, tertiary	19.4 ± 1.2	32 (F)	University students (28% were nutrition majors)	21 ± 2.1	MEQ[b]	35 T/F GN, SS	PT, CV-diet, TR	A 63.7 ± 10.0, C 78.8 ± 9.7	C > A, $p < .0005$
Cupisti, D'Alessandro, Castrogiovanni, Barale, & Morelli, 2002	60 (F)	Mixed, national	14–18	59 (F)	High school students	14–18	SDQ	20 MC GN, SS	PT, IC	A 77.6, C 71.6	A > C, $p < .01$
Frederick & Hawkins, 1992	27 (F)	Track, tertiary	17–25	14 (F)	College students nutrition major	17–25	MEQ[c]	34 T/F GN	PT	A 77.9, C 87.3[d]	C > A, $p < .05$
Guinard, Seador, Beard, & Brown, 1995	20 (M)	Swimming, tertiary	20.2 ± 1.2	20 (M)	University students (not nutrition, exercise majors)	20.5 ± 1.2	SDQ	Other[e] GN	Nil	A 47.3, C 45.2	A > C, $p < .05$
Raymond-Barker, Petroczi, & Quested, 2007	59 (F)	Endurance and trampoline gymnasts, recreational	33.88 ± 9.74	32 (F)	NR	31.7 ± 8.47	EQ[f]	110 Y/N and MC GN	Nil, VQ	A 74.04 ± 11.02, C 68.46 ± 11.75	A > C, $p = .027$
Worme et al., 1990[g]	71 (21 F, 50 M)	Triathlon, recreational	32 ± 2 (F), 39 ± 1 (M)	17 (F), 21 (M)	Medical school or research institute	31 ± 1 (F), 35 ± 2 (M)	SDQ	20 MC GN	IA	C 56.5 ± 2.3, A 54.2 ± 2.0, F 59.4 ± 4.8, M 49.2 ± 5.6	C > A (n.s.), F > M ($p < .01$)
Spendlove et al., 2012	117 (99 F, 76 M)	Elite Australian range of sports	18.9 ± 4.9	84 (F) 32 (M)	Faculty Art students	21.9 ± 4.2	EQ[f]	110 Y/N and MC GN	Nil, VQ	A 57.6 C 63.1	C > A ($p < 0.005$)

Note. F = female; tertiary = college or university teams; NR = not reported; SDQ = self-developed questionnaire; T/F = true/false; GN = general nutrition; SS = sport specific; PT = pretested; CV-diet = content validated by panel including dietitians; MEQ = modified existing questionnaire; TR = test–retest; MC = multiple-choice questionnaire; IC = internal consistency; M = male; nil = no validation/not indicated in the article; EQ = existing questionnaire; Y/N = yes/no statement; VQ = previously validated questionnaire; IA = item analysis. Age is given as $M \pm SD$ or range.

[a]Scores converted to % for easier reader comparison. [b]Werblow et al., 1978. [c]Annable, 1976. [d]A = athletes, C = comparison group, scores reported as $M \pm 0.5$ *SEM*. [e]Other = a questionnaire asking participants to rank 14 foods on high, moderate, or low levels of calories, fat, cholesterol, sugar, salt, fiber, calcium, iron, and vitamins. [f]Parmenter & Wardle, 1999. [g]Values given as $M \pm SEM$.

2.1.6.1 Nutrition knowledge and dietary intake.

The association between nutrition knowledge and dietary intake appears inconsistent and it is likely that multiple factors modify the relationship (Worsley, 2002). One such factor for the inconsistent associations between knowledge and dietary behaviour is that knowledge can be poorly assessed. Despite this, Parmenter and Wardle (1999) stated that, with a well-constructed nutrition questionnaire, strong associations between nutrition intake and knowledge could be identified.

As an athlete becomes elite it can be assumed greater consideration to all factors which can improve performance may be paid (Stoeber et al., 2007); nutrition being one of these. It is often assumed that improving an athlete's nutrition knowledge will result in better dietary choices (Dallongeville et al., 2001) and understanding the reasons that influence food choices may establish an effective way to improve nutritional habit (Dallongeville et al., 2001). Quantifying nutrition knowledge via the use of a questionnaire is a commonly used tool for assessment; however the association between nutrition knowledge and nutritional intake is unclear. A systematic review of nutrition knowledge in athletes (Heaney et al., 2011) found a weak positive association between nutrition knowledge and diet quality (r = 0.44), however the evidence is equivocal. A number of publications have demonstrated a positive relationship (Parmenter and Wardle, 1999, Pirouznia, 2001, Weinsier et al., 1988, Sawyer-Morse et al., 1989, Cho and Fryer, 1974) yet a majority found no significance (Stafleu et al., 1996, Shepherd, 1992, Harrison et al., 1991, Axelson and Brinberg, 1992).

Harrison et al. (1991) investigated nutrition knowledge and dietary habits in 122 elite and non-elite New Zealand athletes, finding that the elite athletes possessed greater nutrition knowledge (67% vs. 58%) and their dietary habits were closer to the NZ nutrition guidelines than those of the non-elite group. A moderate correlation between nutrition knowledge and dietary habits (r = 0.44, p < 0.01) was observed; this relationship was significantly stronger (p < 0.01) among the non-elite athletes (r = 0.62) than the elite athletes (r = 0.23). In non-athletic individuals, Rasanen et al. (2003) reported results from a longitudinal study of individuals who had no nutrition counseling and those that did. The results demonstrated that at the 7-year visit that

people in the counseled group ate less saturated fat and salt than people in the control group; however total fat intake of the two groups did not differ significantly and was greater than the governmental recommend fat intake. Concluding that nutritional counseling influenced the types of fats eaten but did not reduce overall fat intake.

Rand, Pellett and Young (2003) completed a meta-analysis of 235 non-athletic individuals gathered from 19 studies involving nitrogen balance as estimation of protein requirements in healthy adults. The results showed that median estimated average requirement (EAR) for protein was 0.65–0.83g•kg^{-1}•d^{-1}, corresponding to 44.5–58.1g•d^{-1} for an average 70 kg male. As discussed earlier, protein EAR for athletes' can be double that of sedentary adults due to the training and competitive stressors placed on the body, with protein requirements up to 2 g•kg^{-1}•d^{-1}. However it is commonly accepted that some body builders, strength and power athletes consume significantly greater amounts, with reports of athletes frequently consuming > 2.5 g•kg^{-1}•d^{-1} (Forslund et al., 1999). These choices could be due to a number of reasons; (i) the athlete believes what they are doing is correct; (ii) the scientific literature is being ignored by the athlete; (iii) the athlete is unaware of the literature; (iv) correct information is not disseminated to the athletes; (v) incorrect information is being given; (vi) or that the athlete actually thrives on diets with such high quantities of protein. However without any knowledge of correct practise, the implementation of the correct strategy is unlikely (Zinn et al., 2005).

2.1.7 Summary.

To summarise, it has been suggested that athletes may have different nutrition requirements dependent on their phenotype / physiological requirements of the sport. However, current dietary recommendations focus mainly on the demands of the sport rather than the nutrition demands of the individual. The metabolic typing diet is based on a questionnaire which claims to prescribe a diet to meet the specific metabolic requirements of the individual and improve health and performance. This has not been investigated in an athletic population. Furthermore, a weak positive relationship exists between nutrition knowledge and dietary intake, however nutritional intake is commonly reported as inadequate in athletes. It is conceivable that measuring nutrition

knowledge then providing education may result in improved nutrition choices in athletes, although this has yet to be evidenced in UK track and field athletes.

2.2 PART 2 - The cellular regulation of mitochondrial biogenesis and the impact of diet and exercise on metabolic adaptation.

The focus of this thesis is to investigate dietary habits and the impact diet has on some metabolic and cellular processes. The first part of the literature review focused on diet intake and metabolic function. Part 2 investigates the literature surrounding the regulation of mitochondrial biogenesis and the effect of external stressors such as carbohydrate restriction, calorie restriction and exercise on metabolic and cellular responses at rest and during exercise. This provides a rationale for the experimental work detailed later in the thesis.

2.2.1 A brief overview of mitochondrial physiology.

Two main theories exist concerning the origin of the mitochondria. Traditionally it is thought that a eukaryote engulfed the mitochondrion actively via phagocytosis, becoming the first host (Martin and Mentel, 2010). Alternatively, the host that first acquired the mitochondrion may have been a prokaryote. It's origin aside, it is widely agreed that the first mitochondrion containing microfossils dates back 1.45 billion years and have evolved to the ubiquitous organelle and the primary energy producer of the cell we know it to be today (Martin and Mentel, 2010).

The mitochondrion is made of five distinct parts: The outer mitochondrial membrane, the intermembrane space, the inner mitochondrial membrane, the cristae space and the matrix, each plays a specific role in the organelles function. The outer membrane contains a large number of porins that form channels allowing molecules with a molecular weight ≥ 5,000 Daltons to freely diffuse in and out of the mitochondrion (Martin and Mentel, 2010). Due to the permeability of the outer membrane, the inner-membrane space houses concentrations of small molecules such as ions and sugars. Larger proteins such as cytochrome, an essential component of the electron transport chain, are also found in the innermembrane space. The inner mitochondrial membrane

is protein rich and a number of key functions occur within it, including; the redox reactions of oxidative phosphorylation, adenosine triphosphate (ATP) synthase and mitochondrial protein fusion and fission. Encapsulated within the inner mitochondrial membrane are numerous cristae, the folded shape of the cristae enhance the surface area of the inner mitochondrial membrane, enabling an enhanced production of ATP. Situated inside the inner membrane is the matrix, this space contains approximately 66% of total mitochondrial protein. The matrix is essential to the production of ATP as it contains a concentrated mixture of hundreds of enzymes that play a key role in substrate utilisation and the citric acid cycle, as such is vital for energy production during exercise. With the mitochondria being demonstrated to have such a key role in energy metabolism, a significant body of work has been focused around the manipulation of mitochondrial biogenesis to help cure diseases (Lee et al., 1998) through to enhancing sporting performance (Wright et al., 2007). During daily function, sustained and prolonged ATP production is an essential requirement due to limited stores of ATP within the skeletal muscle (Greenhaff et al., 1994), the mitochondria primarily fulfils the role of ATP producer. With the high mechanical workload of the skeletal muscle, muscle fibres are densely populated with mitochondria to meet the energy demand.

Mitochondria are responsible for energy production via fatty-acid oxidation, β oxidation, Krebs cycle, and oxidative phosphorylation. Increased mitochondrial quantity and density have been linked with increased endurance performance (Psilander et al., 2013), reduction in type 2 diabetes, increased resisting metabolic rate (Larsen et al., 2011) and improved insulin sensitivity (Lee et al., 1998, De Feyter et al., 2008, Patti et al., 2003, Petersen et al., 2004, Mootha et al., 2003), promoting both health and exercise performance benefits. The process of mitochondrial biogenesis requires the co-ordinated synthesis of > 1,000 proteins encoded by the nuclear genome, with a contribution from mitochondrial DNA, and can be defined as the growth or division of pre-existing mitochondria (Jornayvaz and Shulman, 2010). To maintain health, mitochondria engage in several dynamic processes, including fusion (the joining of two organelles into one), fission (the division of a single organelle into two), transport (directed movement within a cell), and mitophagy (targeted and programmed destruction) (Mishra and Chan, 2016) (Figure 2.2.1 and Figure 2.2.2). In addition to the complex synthesis, the metabolic control of mitochondrial biogenesis is impacted

by a number of environmental stressors such as exercise, calorie restriction, low temperature and hypoxia leading to a dynamic state of biogenesis (Jornayvaz and Shulman, 2010).

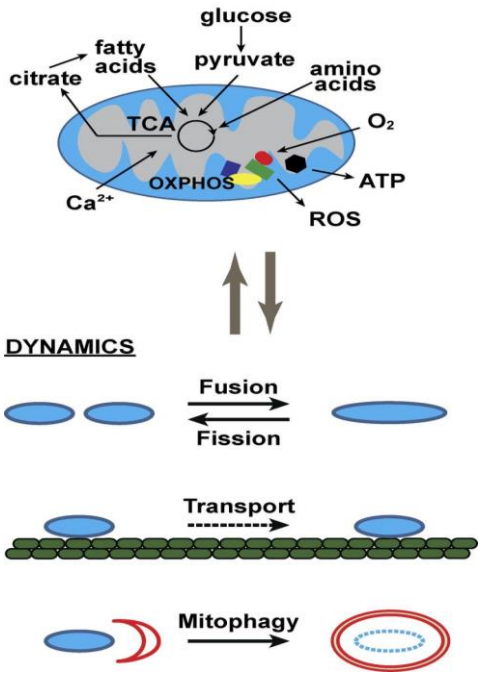


Figure 2.2.1. Illustrative representation of mitochondrial fusion, fission, transport and mitophagy.

The biochemical pathways which impinge the mitochondrial metabolism and dynamics (Mishra and Chan, 2016).

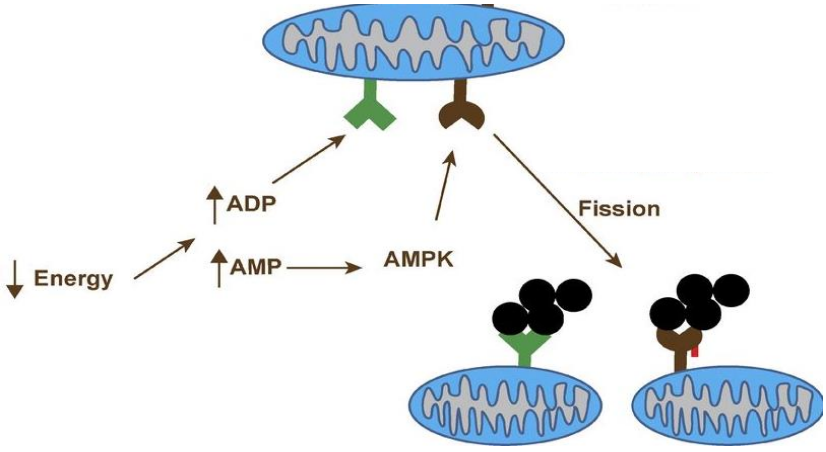


Figure 2.2.2. Metabolic regulation of mitochondrial fission.

Severe energy depletion can activate fission via elevation of ADP and AMP levels. AMP-sensing by AMPK results in phosphorylating a number of receptor proteins initiating mitochondrial fission. (Adapted from Mishra and Chan (2016).

2.2.2 Skeletal muscle metabolic control through the PGC-1α family of transcription coactivators.

A seminal paper from Puigserver et al. (1998) first isolated PGC-1-α and demonstrated its key role in the regulation of mitochondrial processes linked to adaptive thermogenesis. In the subsequent years the fundamental functions of PGC-1α in relation to the regulation and co-activation of mitochondrial biogenesis has been further demonstrated, and it is now well established that the PGC-1α plays a crucial role in the regulation of mitochondrial biogenesis (Liang and Ward, 2006)

The gene of the PGC-1α protein is located on chromosome 4 in humans and encodes a protein containing 798 amino acids. PGC-1α has two putative nuclear localisation signals and is located in the cell nucleus (Puigserver et al., 1998). It is found at high levels where mitochondria are abundant and oxidative metabolism is highly active (Liang and Ward, 2006). PGC-1α works as a co-transcriptional regulation factor that induces mitochondrial biogenesis by initiating transcriptional coactivators. It also has a crucial role in linking stimuli to induce internal metabolic response such as: adaptive thermogenesis, glucose/fatty acid metabolism and fibre type switching in skeletal muscle and heart development (Puigserver et al., 1998). The stimulation of mitochondria genes, by PGC-1α, leads to increased enzymatic capacity for fatty-acid, β oxidation, Krebs cycle, and oxidative phosphorylation. Furthermore deficiency of PGC-1α mitochondrial numbers decreases respiratory capacity in oxidative muscle fibres and reduces exercise capacity and fatigue index (Leone et al., 2005).

PGC-1α acts as a coactivator of a number of transcription factors (Table 2.2.1), of which the nuclear receptor hormones PPARα (Vega et al., 2000) and nuclear respiratory factor-1 (NRF-1) (Wu et al., 1999) command particular attention with regards to mitochondrial synthesis. PPARα is enriched in tissues with high oxidative energy demands dependent on mitochondrial fatty acid oxidation, and PPARα interacts with PGC-1α to coactivate target genes involved in mitochondrial fatty acid oxidation (Vega et al., 2000). In a 3T3-L1 pre-adiposities cell line and following a 6-hour incubation in palmitate, Vega et al. (2000) demonstrated that over expression of

both PGC-1α and PPARα resulted in increased mitochondrial flux through the fatty acid oxidation pathway. With regards to NRF-1, in C2C12 muscle cell line the presence of PGC-1α and NRF-1 increased the content of mitochondrial DNA by 80% over the control (Wu et al., 1999), demonstrating the essential role PGC-1α plays in regulation of mitochondrial biogenesis via NRF-1 and fatty acid oxidation with the PPARα pathway.

Table 2.2.1. Selected list of transcription factors for which PGC-1α functions as a coactivator.

Transcription Factor	**Function**
NRF1	Mitochondrial biogenesis
NRF2	Mitochondrial biogenesis
ERR-α/β/γ	Mitochondrial biogenesis
PPAR	Fatty acid oxidation
TR-β	CPT-I induction
FXR	Triglyceride metabolism
GR	Gluconeogenesis
HNF-4α	Gluconeogenesis
FOXO1	Gluconeogenesis

PPAR = proliferator peroxisome-acitvated receptor, NRF = nuclear respiratory factor, ERR = estrogen-related receptor, TR = thyroid receptor, CPT-1 = carnitine palmitoyltransferase-I, FXR = farnesoid X receptor, GR = glucocortoid receptor, HNF = hepatic nuclear factor, FOXO1 = forkhead box O1 (Adapted from Liang and Ward (2006))

PGC-1α phosphorylation occurs via several different pathways; AMP-activated protein kinase (AMPK) and silent information regulator T1 (SIRT1) mediated deacetylation (Gurd et al., 2009) have been demonstrated to regulate the expression of PGC-1α in human muscle (Ruderman et al., 2010), both of which are stimulated by environmental stressors. It is important to note that PGC-1α shares similar molecular structures and functions as PGC-1β (binding sites and transcriptional activation), but PGC-1β also plays a role in regulating mitochondrial biogenesis. However, unlike PGC-1α, PGC-1β is not up-regulated in brown adipose tissue in response to cold, nor in the muscle in response to exercise (Meirhaeghe et al., 2003), suggesting that PGC-1α and PGC-1β are stimulated independently.

AMPK is a fuel sensing enzyme that is activated by a decrease in the cell's energy availability, reflected by an increase in AMP/ATP ratio (Ruderman et al., 2010). When a cell is energy stressed an increase in liver kinase B1 (LKB1) and Ca^{2+} occurs, which can in turn and independently catalyze AMPK phosphorylation by altering the AMP/ATP ratio. AMPK's activation initiates metabolic and genetic processes, such as fatty acid metabolism, which help to restore body ATP stores. Additionally, as AMPK is a primary upstream initiator of mitochondrial biogenesis, increases in AMPK can chronically increase the energy production capacity of the cell (Jager et al., 2007).

SIRT1 is widely expressed in a range of mammalian tissues including liver, skeletal muscle, adipose tissue, pancreas, brain and endothelium (Ruderman et al., 2010). With reference to skeletal muscle, SIRT1's regulation is not a clear as AMPK. However changes in SIRT1 have been attributed to increased NAD^+ and NAD^+/NADH ratio, with reduced nutrient availability (Cohen et al., 2004) and energy expenditure (Suwa et al., 2008) initiating a response in SIRT1.

Ruderman et al. (2010) suggest that an AMPK/SIRT1 axis exists, and hypothesise that concurrently AMPK and SIRT1 may initiate the simultaneous activation of each other. An increase in the NAD^+/NADH ratio initiates the expression of SIRT1, which in turn deacetylates and activates LKB1, activating AMPK. The activation of AMPK, by energy deprivation or other means, may increase the NAD^+/NADH and/or the activity of Nampt resulting in the activation of SIRT1 (Fulco et al., 2008) (Figure 2.2.3). Either way, independently or concurrently, AMPK and SIRT1 are well established as upstream activators of PGC-1α, which in turn has been demonstrated as the primary regulator of mitochondrial biogenesis (Liang and Ward, 2006).

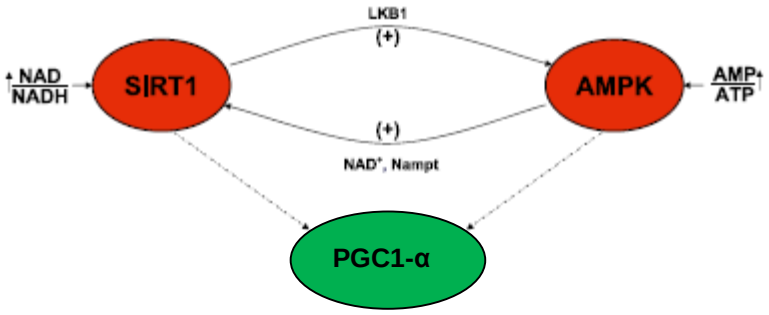


Figure 2.2.3. The theoretical AMPK/SIRT1 – PGC-1α axis.

(Adapted from Ruderman et al. (2010)).

2.2.3 The regulation of PGC-1α through eternal stressors.

A substantial body of evidence suggests that SIRT1 expression responds to both increases and decreases in nutrient availability (Nemoto et al., 2005), along with increases in energy expenditure (Suwa et al., 2008) (Figure 2.2.8). Additionally, AMPK is stimulated by cellular stress that causes a depletion of ATP and elevation of AMP, similar stressors such as calorie restriction (Itani et al., 2003), hypoglycaemia (Itani et al., 2003) exercise (Fujii et al., 2000) and muscular contraction (Hutber et al., 1997) results in increased AMPK expression (Jager et al., 2007). As such, increases in PGC-1α mRNA expression have been observed in response to calorie restriction, hypoglycaemia and endurance exercise (Itani et al., 2003) resulting in positive metabolic perturbation. Due to the importance of mitochondria in metabolism and the role of PGC-1α in mitochondrial biogenesis and subsequent energy production, a number of nutrition and exercise strategies have been employed to increase the expression of PGC-1α, and consequent mitochondrial biogenesis.

Energy reduction (fasting) is a potent stimulator of metabolic adaptation. Canto et al. (2010) demonstrated this in a series of well-designed experiments. Following either a 20-hour fast or normal fed diet, wild type (WT) and AMPKγ_3 KO mice were sacrificed and quadriceps muscle tissue was extracted. A >2-fold increase ($p < 0.05$) was observed in relative PGC-1α mRNA levels along with a significant reduction in PGC-1α acetylation levels in the WT mice (Figure2.2.4), highlighting both an increased

expression and activity of PGC-1α in response to restricted energy availability. Additionally, Canto et al. (2010) observed no change in PGC-1α mRNA levels or acetylation in either the fed or fasted state in the AMPKγ$_3$ KO mice, demonstrating the key role AMPK plays in the activation of PGC-1α.

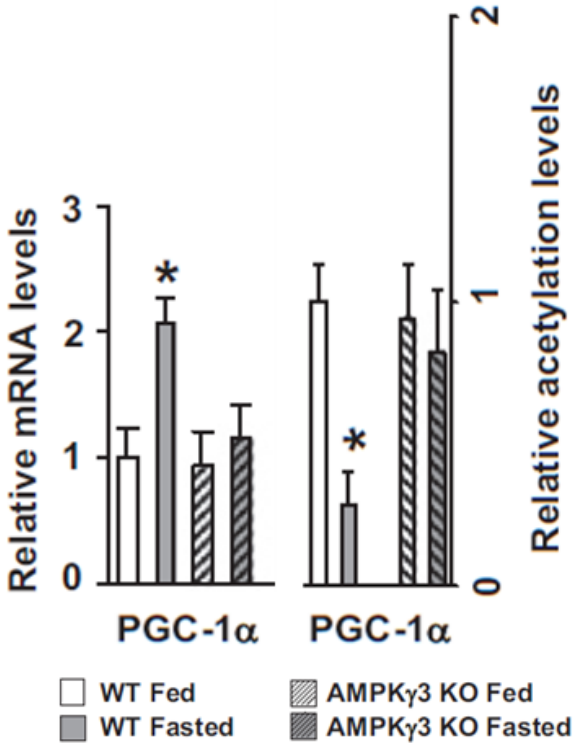
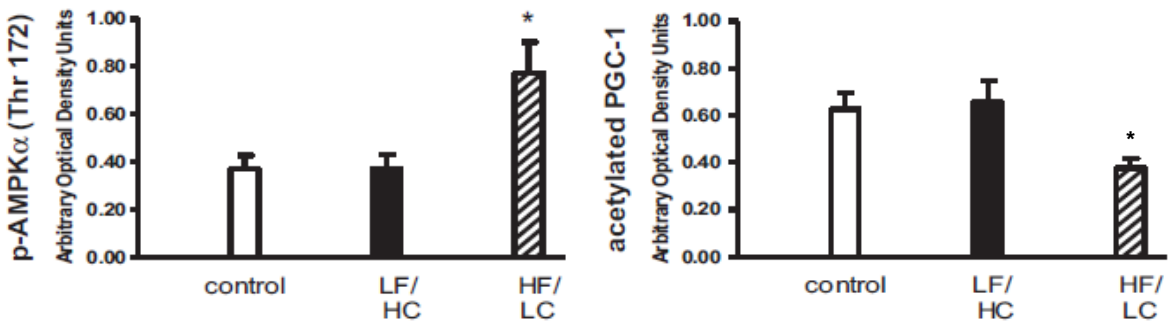


Figure 2.2.4. PGC-1α deacetylation and transcriptional response to fasting in WT and AMPKγ$_3$ KO mice.

*Fasting increase PGC-1α deacetylation and mRNA transcriptional response in WT mice, the impact of fasting is negated in AMPKγ$_3$ KO mice. * indicates statistical significance from relative fed group (adapted from Canto et al. (2010))*

Draznin et al. (2012) published a dual study investigating the effect dietary macronutrient composition had on AMPK and SIRT1 expression and activity in human skeletal muscle. The participants in study one were 21 healthy and lean (men = 11, women = 10) individuals, where as the cohort in study two consisted of 18 obese participants (men = 8, women = 10). Baseline data was collected then the participants were randomly assigned to a dietary intervention; the lean group were placed on either a low-fat/high-carbohydrate (LF/HC; 20% fat, 60% carbohydrate, 20% protein eucaloric diet), a high-fat/low-carbohydrate (HF/LC; 50% fat, 30% carbohydrate, 20% protein eucaloric diet) or overfeeding (40% calorific excess); the obese group were

assigned to the same diet ratio, but the diets were hypocaloric (30% calorie restriction). In study one, the lean participants showed no significant changes in body weight, insulinemia, glycemia or observed lipid levels across the three conditions after 5-days dietary manipulation. Total AMPK, SIRT1 and PGC-1α had no change, however AMPK phosphorylation and PGC-1α deacetylation significantly increased ($p < 0.05$) in the HF/LC group (Figure 2.2.5 A and B). In the obese group modest weight loss was observed, and fasting insulinemia was significantly reduced in both groups. The HF/LC hypocaloric diet significantly increased AMPK phosphorylation and PGC-1α deacetylation, however in contrast the LF/HC hypocaloric diet significantly reduced AMPK phosphorylation and PGC-1α deacetylation, suggesting high carbohydrate intake prevents the activation of the AMPK-SIRT1 axis in skeletal muscle that would otherwise be activated by a low calorie diet.



A **B**

Figure 2.2.5. Effect of hypocaloric diets on AMPK phosphorylation and PGC-1α acetylation.

*Effect of a hypocaloric diet containing either low fat / high carbohydrate (HF/LC) or high fat / low carbohydrate (HF/LC) in obese individuals on phosphorylation of AMPK (**A**) and acetylation of PGC-1α (**B**). * indicates statistical (p < 0.05) significance from baseline (adapted from Draznin et al. (2012)).*

The impact of a single bout of endurance exercise on PGC-1α mRNA expression is fairly well explored and robust (Hawley and Morton, 2014). Additionally the effect of endurance exercise independent to, and in combination with glycogen restriction on PGC-1α expression and phosphorylation is well researched. Psilander et al. (2013) demonstrated that in a normal glycogen state (478 ± 33 mmol•kg^{-1} dry weight muscle) 3-hours post a 60 minutes interval session (mean $\dot{V}O_2$max 65 ± 1 mL•kg^{-1}•min^{-1}) PGC-1α expression was increased 2.5 fold. Whereas in a low glycogen (166 ± 21 mmol•kg^{-}

[1] dry weight muscle) state an 8.1 fold increase was observed (Figure 2.2.6); clearly demonstrating the role exercise alone and exercise in combination with restricted glycogen availability has on PGC-1α expression.

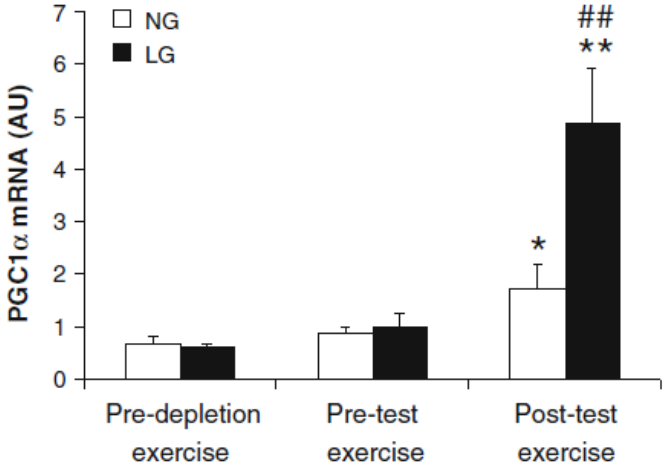


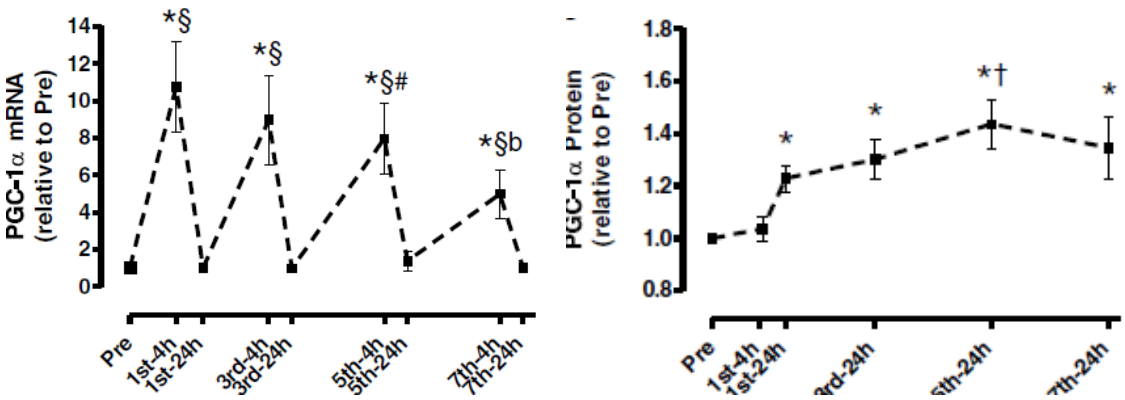
Figure 2.2.6. PGC-1α mRNA expression.

PGC-1α mRNA expression (arbitrary units) 3-hour post 60 minute high intensity interval cycling in either normal glycogen (NG) or low glycogen (LG) condition (Psilander et al., 2013).

However in that study it is important to consider, that the low glycogen group had a calorie deficit of 1,425 kcal relative to the normal glycogen group, also glycogen restriction was reached through a significant increase in dietary fat rather than protein. Fat intake increased from 6% in the high glycogen group to 77% in the low glycogen group. As discussed earlier in this Chapter calorie restriction is also a potent stimulator of PGC-1α, thus the calorie deficit may be a confounding factor in the conclusions of this study. However, a similar fold response in PGC-1α mRNA expression was observed following high intensity internal training in a fed state. Following 4 x 30 second all out sprints (4 minutes rest between) Gibala et al. (2009) demonstrated an ~2-fold ($p < 0.05$) increase in PGC-1α mRNA expression 3 hours post exercise which supports that data observed from Psilander et al. (2013)

To date very limited literature exists exploring the time course response of PGC-1α mRNA expression post exercise. Baar et al. (2002) investigated the adaptive response of skeletal muscle during and subsequent to a five day endurance swimming

intervention (2 x 3-hour swimming bouts with 45 minutes rest) in rats accustomed to swimming. Baar et al. (2002) demonstrated a two-fold increase in PGC-1α mRNA expression in the triceps 6 hours post exercise, PGC-1α protein concentration was also increased two-fold 18-hours post exercise. A similar magnitude in PGC-1α protein was observed 18 hours after the fifth day of the exercise protocol. This is an important finding because increases in mRNA do not always result in a parallel increase in protein, demonstrating that an increase in the expression of PGC-1α mRNA results in an equivalent response in protein content.



A **B**

Figure 2.2.7. Time course response of skeletal muscle PGC-1α mRNA and protein content.

*Time course skeletal muscle PGC-1α mRNA (**A**) and protein content (**B**) throughout 2 weeks of high-intensity interval training. (mean ± SEM). *Significantly different from Pre, §significantly different from all 24 h time points, #significantly different from 1st-4 h, †significantly different from 1st-24 h (p < 0.05) (adapted from Perry et al. (2010)).*

Perry et al. (2010) published a study investigating the transcriptional and mitochondrial protein response in untrained males (mean $\dot{V}O_2$peak of 45.5 mL•kg^{-1}•min^{-1}) throughout a two-week period (7 sessions) of high-intensity interval training. PGC-1α mRNA increased by >10-fold within four hours after the initial exercise session and returned to pre exercise levels 24h into recovery (p <0.05; Figure 2.2.7 A). Each subsequent session repeatedly stimulated increases in PGC-1α mRNA 4-hours after the session followed by a return to the pre level by 24 hours (p <0.05). However, the magnitude of the 4-hour increase after each training session was progressively decreased.

Conversely, PGC-1α protein expression occurred later (23%, 24-hours after the 1st session, p <0.05; Figure 2.2.7 B) than PGC-1α mRNA. Unlike the reduced PGC-1α mRNA responses observed over seven training sessions, PGC-1α protein expression continued to increase, attaining a plateau (+42%, p <0.05) by the 5–7th training session.

This time course data may offer some insight into transcriptional response post exercise, however it is important to consider that the participants were untrained and exhibited an increase in $\dot{V}O_2$peak of 5.4 mL•kg^{-1}•min^{-1} after completing seven training sessions in two weeks. Such significant improvement following just seven training sessions would not be observed in well trained individuals, more research is needed to investigate the response in well trained individuals.

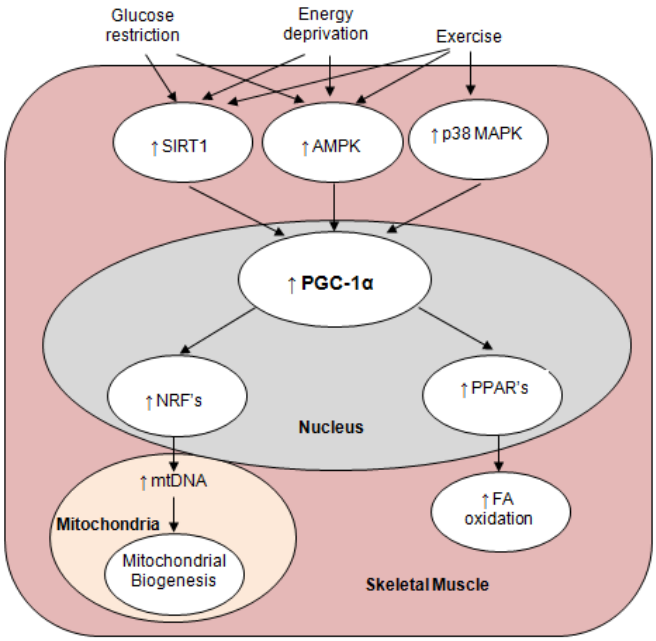


Figure 2.2.8. The activation of PGC1-α and its role in functioning as a co-activator.

Energy deprivation, glucose restriction and exercise increase SIRT's, AMPK and p38 MAPK expression which, in turn, causes an increase in PGC-1α gene transcription. This increase NRF activity leading to increased mitochondrial DNA (mtDNA) and protein expression thus promoting mitochondrial biogenesis. An increase in PGC-1α also results in an PPAR activity and greater fatty acid (FA) oxidation. (Adapted from Jornayvaz and Shulman (2010)).

As previously discussed it is well established that glycogen availability is a key factor in the regulation of PGC-1α and a large number of athletes anecdotally follow a high protein diet, thus restricting carbohydrate availability. To date, with regards to the AMPK/SIRT1 – PGC-1α metabolic pathway, most the research in humans used fat as the macronutrient manipulated to reduce carbohydrate intake and maintain energy availability; protein intake is kept consistent. In rats, Nakazato and Song (2008) investigated the impact a high protein diet has on oxidative properties and PGC-1α in the gastrocnemius muscle. In three matched groups, 19 rats (four weeks old) were fed either a control (15% protein, 71.3% carbohydrate), mid protein (25% protein, 41.3% carbohydrate), or a high protein (35% protein, 31.3% carbohydrate) diet for four weeks. Following this the rats were killed and target tissues were removed and frozen. Energy intake across the three groups was isocaloric; however the high protein group demonstrated a significantly lower body mass, body fat and skeletal muscle mass than the control group. Importantly the high protein group exhibited a greater expression of both succinate dehydrogenase and PGC-1α ($p < 0.05$) expression relative to the control group. These differences in oxidation potential were only observed in muscle fibres that were originally sparse of oxidative muscle tissue. Unfortunately Nakazato and Song (2008) did not report the levels of coactivators, such as AMPK and SIRT1, however it can be speculated that high protein—low carbohydrate diets mediate insulin response to feedings along with a reduction in blood glucose, providing a stimulus similar to that of hypoglycaemia and calorie restriction, which has previously been demonstrated to increase the AMPK/SIRT1 axis.

2.3 Protein metabolism in a negative energy balance.

Skeletal muscle mass is maintained by a complex cascade of intracellular signalling pathways, which are known to be influenced by energy availability, exercise, and nutrient availability. In response to a negative energy balance, mRNA translation and MPS may become down regulated as a result of lower insulin (growth factor) and reduced energy availability (which as discussed previously Chapter 2.2.2, increases the AMP/ADP ratio), as such causing myofibrillar degradation and decrease MPS, and subsequent skeletal muscle mass (Figure 2.3.1) (Carbone et al., 2012).

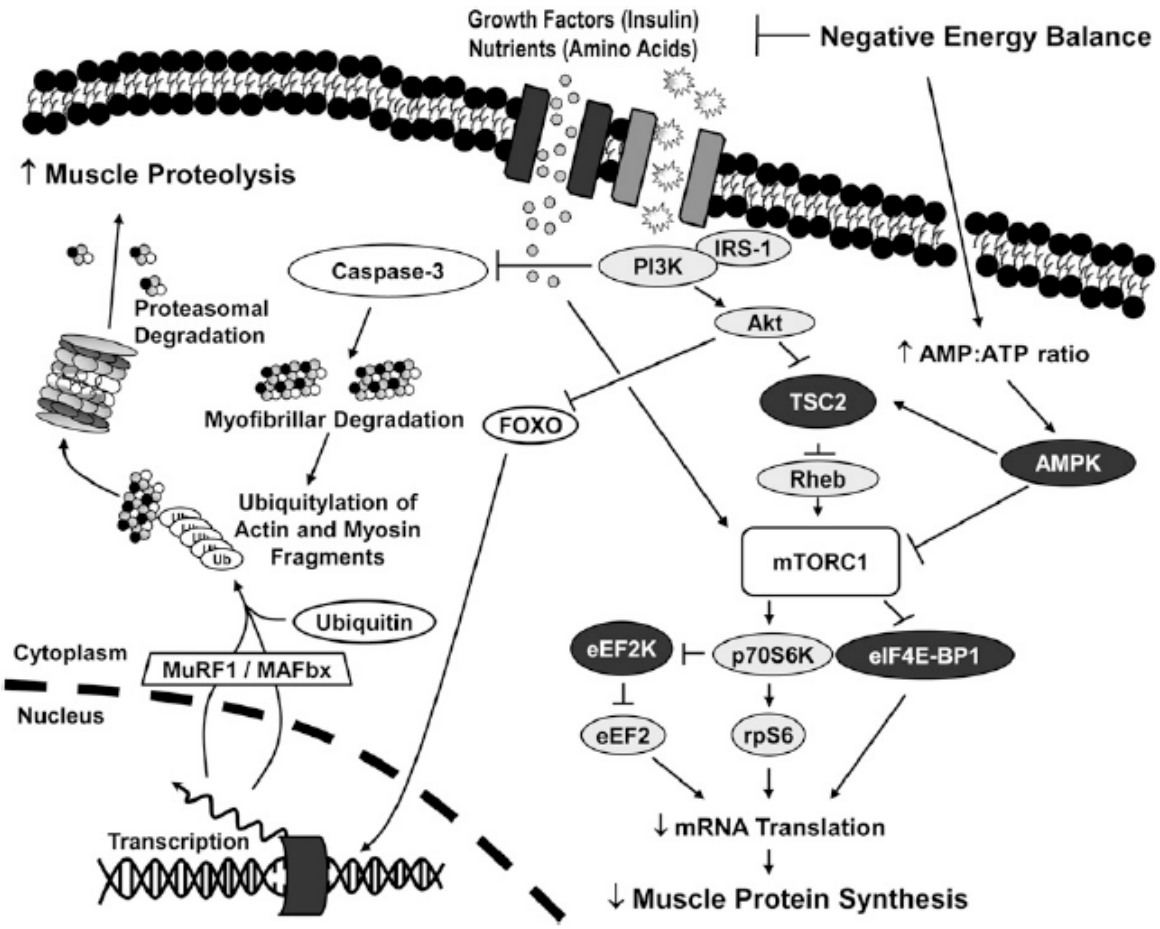


Figure 2.3.1. Role of negative energy balance and reduced growth factors on MPS *(Carbone et al., 2012)*

The consequences of negative energy balance on total body and skeletal muscle mass are well established. In general, total body mass decreases in response to sustained (> 5-days) periods of negative energy balance, and the proportion of body mass loss is ~70% adipose tissue and 30% fat-free mass (Weinheimer et al., 2010). Layman et al. (2003) reported a greater fat mass loss and improved maintenance of fat free mass (fat: lean ratio (g^{-1}•g^{-1}) in high protein group 6.3 ± 1.1 vs. 3.8 ± 0.9 in high carbohydrate group) in overweight women on a high protein, hypocaloric (1.6 g•kg^{-1}•day^{-1}) compared to a protein diet matched for RDA (0.8 g•kg^{-1}•day^{-1}). The effect of a hypocaloric diet on body composition is important, a diet which is matched for total weight loss but results in a greater muscle mass atrophy may down-regulate resting metabolic rate and protein turnover (Ravussin et al., 1988, Stein et al., 1991), thus compromising health and metabolism, as such diets which maintain skeletal muscle mass are deemed preferential (Carbone et al., 2012). Several studies have described

the beneficial impact of consuming diets with a higher protein intake on muscle–sparing (Skov et al., 1999, Rodriguez and Garlick, 2008). Although the literature provides useful evidence, the exact mechanisms of increasing protein intake to maintain skeletal muscle mass in dietary weight loss programmes is yet to be fully understood.

Reducing energy availability (and carbohydrate intake) may be beneficial to the adaptive response for the AMPK/SIRT1 – PGC-1α axis (Figure 2.2.8), however this may have a detrimental impact on the maintenance of skeletal muscle mass through the mTORC1 pathway (Figure 2.3.1), and compromising skeletal muscle mass. Also, increasing dietary protein intake in a calorie deficient state has been demonstrated to attenuate the catabolic response (Skov et al., 1999), which raises the question; does a high protein diet in combination with endurance exercise have beneficial impact on lean mass maintenance and the adaptive transcriptional pathways responsible for improvements in endurance performance?

2.3.1 Nutrition manipulation to enhance endurance training adaptation in skeletal muscle

It is well established that carbohydrate availability is rate limiting to endurance performance (Bergstrom et al., 1967). Endogenous carbohydrate stores are only sufficient to fuel approximately three hours of sub-maximal performance (Burke and Hawley, 2002), where as fat stores, in the form of intra-muscular triglycerides, blood lipids and adipose tissue, in even the leanest athlete, are sufficient to provide enough fuel for numerous days of low intensity activity (Rapoport, 2010). Some early work from Coyle et al. (1986) demonstrated that well trained athletes have a greater ability to oxidise fat during prolonged moderate intensity exercise. Hawley et al. (1998) progressed the research area with a paper discussing strategies to enhance fat utilisation during exercise; and many “popular” diet books claimed that “fat loading” strategies would enhance performance capabilities of endurance and ultra-endurance athletes by making them better able to “tap into body fat stores” (Sears, 1995). In the 1980’s and 90’s a number of research papers were published highlighting the possibility of a high fat diet improving endurance performance by enhancing the fat

oxidation pathway (Phinney et al., 1983, Goedecke et al., 1999, Lambert et al., 1994). However a review paper by Burke and Hawley (2002) stated:

> "It is perplexing that in the face of marked changes in metabolism that favour fat oxidation and the consequent sparing of muscle glycogen, fat-adaptation/CHO restoration strategies do not provide clear benefits to the performance of prolonged exercise."

To the author's knowledge a total of eleven studies have been published investigating the impact of continuous multi-day (5-days to 28-days) low carbohydrate vs. high carbohydrate diets on either high-intensity or sub-maximal endurance performance in trained athletes. It is important to note that study design, feeding during and performance measured differed throughout. In summary, with regards to high-intensity exercise, five studies observed no change in performance, five showed that low carbohydrate decreased performance (one was inconclusive, suggesting individual response) and one showed an improvement (however this was subsequent to 150 minutes sub maximal cycling). With regards to sub-maximal endurance performance nine studies demonstrated no change and two studies showed an improvement (Table 2.3.1). All studies highlighted above manipulated fat to restrict carbohydrate intake, and classify the study design as low carbohydrate high fat (LCHF) (protein intake is generally not referenced or not changed). Furthermore, all literature to date is on cyclists (Havemann et al., 2006, Lambert et al., 1994, Goedecke et al., 1999, Rowlands and Hopkins, 2002, Vogt et al., 2003, Burke et al., 2002, Carey et al., 2001, Noakes, 2004, Burke et al., 2000, Lambert et al., 2001, O'Keeffe et al., 1989).

To date, only three studies have reported an improvement in exercise performance following a 'train low' strategy. Hansen et al. (2005) used a 10-week unilateral isokinetic leg kicking protocol in which one leg undertook 50% of sessions with lowered glycogen (a twice a day training program with the second session commencing after glycogen depletion from the first session) and the other leg undertook the same training but glycogen was restored in-between sessions. The results reported a greater increase (19.7 ± 2.4 min vs. 11.9 ± 1.3 min) in isokinetic leg kicking time to exhaustion in the lowered glycogen leg. However with the outcome variable being an isolated movement, with little application to sporting performance, the improvements in

performance observed should be taken with caution when relating to full body exercise performance.

Table 2.3.1. Impact of a high fat, low carbohydrate diet on exercise performance. *(adapted from Burke (2015))*

Intensity	Impact of a low carbohydrate (high fat) diet on exercise performance		
	Improve	**No Change**	**Decrease**
Sub maximal	2	9	0
High intensity	1	5	4

Cochran et al. (2015) investigated the effect of restricted carbohydrate availability between twice-a-day high-intensity training on a cycling time trial and repeated sprint test. Active, male participants (*N* = 18, mean $\dot{V}O_2$peak = 44 ± 9 ml•kg^{-1}•min^{-1}) were provided either a high carbohydrate (195 g carbohydrate) or low carbohydrate (17g carbohydrate) meal in the three hours between training sessions 6-days a week over a two week training period. The training-induced improvement of mean power output during a 250-kJ TT performance was greater (p = 0.02) in the group that undertook the second session with reduced CHO availability (PRE, 211 ± 66 W; POST, 244 ± 75 W) compared with the group that refuelled before that session (PRE, 203 ± 53 W; POST, 219 ± 60 W). It is important to note that there was no difference between groups in the improvement in the repeated sprints test (5 × 15s all out sprints) or mitochondrial content. Another important consideration is the training status of the individuals, as with the Perry et al. (2010) study, the participants were untrained individuals, as such the amplitude of response is expected to be greater than in well trained individuals (Ahtiainen et al., 2003).

Utilising a unique study design Marquet et al. (2016) investigated the impact of 3-week sleeping with low carbohydrate availability, compared to regular daily carbohydrate intake, on simulated Olympic distance triathlon performance. The sleep low group completed a high-intensity workout with high carbohydrate availability the night before, followed by an overnight fast and a prolonged submaximal workout the in the morning. The control group completed the same training schedule but with normal carbohydrate

intake. No difference in total carbohydrate intake was observed between groups. All the sleep low participants reduced their 10km time trial performance following the intervention (pre, 40:23 minutes ± 3:22; post, 39:10 minutes ± 3:02, $p < 0.01$), whereas no change was observed in the control group (pre, 41:26 minutes ± 2:13; post, 41:24 minutes ± 2:43), also a significant reduction ($p < 0.001$) in body mass was also observed in the sleep low group. Due to the 'real world' nature of the study design no invasive mechanistic markers were assessed, however this further highlights that carbohydrate is a potent mediator in the adaptive response to endurance training (Hawley and Morton, 2014, Marquet et al., 2016).

2.4 Key methodological considerations.

2.4.1 Measuring Dietary Intake

Measuring dietary intake is key to understanding what foods an individual is consuming; however there is no truly accurate measure of dietary intake in free-living people (Burke and Deakin, 2010). A wide range of analysis tools/methods have been developed, each with their own strengths and limitations (Magkos and Yannakoulia, 2003). This literature review will not provide an in-depth analysis of each method, however Table 2.4.1 provides a summary of the primary dietary analysis methods used in research. For further reading please refer to the excellent articles from Magkos and Yannakoulia (2003) and Burke and Deakin (2010).

Due to its flexibility, compliance and accuracy, estimated method using household measures is generally seen as the most feasible method for research (Bingham et al., 1994). Table 2.4.2 highlights the common methods used in an athletic population (Burke et al., 2001). The 2–4 day estimated method using household measures was the most popular method with 44.5% or research studies utilising the technique. In total 76.7% used the estimated using household measures method over 1–12 days. The accuracy of estimated diet records are deemed sufficient for the assessment of groups, and a 3-day record including 2-week days and 1-weekend day meets the requirements to assess individual intake (Magkos and Yannakoulia, 2003). Recording periods of > 6-days have been used, however these are usually unsatisfactory due to respondent fatigue (Thompson and Byers, 1994). Additionally, when assessing the dietary intake of groups fewer days are required compared to individual analysis. Basiotis et al. (1987) states that 14-days diet record are required to estimate the true average intake of an individual, in comparison to just 3-days when calculating for groups.

Table 2.4.1. Overview of different methods for dietary assessment.

(adapted from Burke and Deakin (2010)).

Data Collection Method	Application	Strengths	Limitations
Weighed method using scales or computerised approaches	Assess food choices and eating habits mostly from 1 to 7-days	• Weighing is considered an accurate method	• Time-consuming to conduct and analyse • Requires trained personnel • Requires literate and co-operative respondents
Estimated method using household measures	Acceptable for research because better compliance than weighed method	• Provides information about eating habits • Fairly valid for up to 5-days • Provides detailed information	• Poor compliance after 4-days • Not representative of usual diet, unless repeated • Under estimates energy from 20 – 50 %
Duplicate food collections	Uses duplicate meals/foods for direct chemical analysis	• Most accurate method	• High respondent burden • Expensive to analyse • Distorts food choice • Under-reporting • Other biases poorly documented
24-hour recall	Used mainly to rank food or nutrient intake of groups of people Can be used to rank food and nutrient intakes of individuals, if repeated at random	• Minimal distortion of food intake • Low respondent burden • Good response rate • Low administration cost	• Memory/recall bias • Under estimates total energy intakes
Food frequency questionnaires (FFQ's)	Mainly for ranking usual food or nutrient intakes of groups of people in qualitative or semi quantitative terms As screening tool to detect, measure or rank specific nutrients or food intakes in groups or individuals	• Similar advantages to the 24-hour recall • Measures usual diet and may be more representative of 'usual' intake than repeated diet recall. • Quick to administer • Cost effective	• Similar limitations to 24-hour recall • Less accurate than record methods • List of foods may not fully represent foods consumed by respondents • Difficulty quantifying portion sizes. • Over-estimation at low intakes, under estimation at high intakes.
Dietary history	Combines a 24-hour recall and FFQ Assessment of usual intakes of individuals in clinical practice	• Comprehensive assessment of the usual nutrient intake, including seasonal changes	• Time consuming to conduct • Dependent on highly trained interviewer • Dependent on memory and co-operation of the respondent • Tend to over-estimate nutrients intake

Table 2.4.2. Dietary analysis method used in studies involving athletes.

(adapted from Burke et al. (2001)).

Dietary method	Number of days	Number of population groups assessed	Percentage of studies
Household measures	1 to 3	5	2.7
	2	4	2.1
	3	66	34.9
	4	14	7.4
	4 to 7	20	10.6
	5	4	2.1
	6	2	1.1
	7	27	14.3
	9	1	0.5
	10	1	0.5
	12	1	0.5
Weighed	3	4	2.1
	3 to 5	7	3.7
	4	3	1.6
	5	2	1.1
	7	6	3.2
Dietary recall	7	1	0.5
24h recall	N/A	6	3.2
FFQ	N/A	2	1.1
Diet history	N/A	6	3.2
Semi weighed	3	7	3.7

It is important to note that all dietary records and surveys are opposed by errors of validity (accuracy of data collection) and reliability (how well the data reflects intake). The three most common errors in self-reporting diaries are highlighted as:

- The individual may alter their dietary intake during this period, therefore not representing usual intake.
- The individual may record a false diary, omitting or adding foods in an attempt to make their diet look of better quality.
- The individual may make fundamental errors in recording portion sizes and weights. (Burke et al., 2001).

Mertz et al. (1991) investigated the relationship between estimated energy intake and actual dietary intake for body weight maintenance. A cohort of 263 free-living humans completed a minimum of 7-days food record training from a dietician, followed by a controlled diet of conventional foods for ≥ 45 days. The participants were required to estimate food consumed using the household measures method. The results showed on average 18% under reporting of calorie consumption (8% overestimated, 11% were accurate to within 100 kcal and 81% under reported), however interestingly the individuals who had a greater calorie intake estimated food intake more closely to actual intake. With the populations of the current research being athletes, presumably with higher calorie consumption, it could be hypothesised that the results are a more accurate representation of actual intake relative to a non-athletic population (Mertz et al., 1991). However (Magkos and Yannakoulia, 2003) reported a mean underestimation of ~28 % relative to energy expenditure in athletes, but stated that macronutrient ratios were representative of dietary intake.

Although no truly accurate method exists to measure dietary intake in free-living individuals, measuring nutrition intake is a vital tool when assessing current habits of an athlete. However when analysing, it is important to consider the potential errors of measurement.

2.5 Summary of literature and rationale for research

It is well established that optimal nutritional intake can enhance both health and endurance performance. It is commonly reported that athletes dietary intake is nutritionally inadequate (Heaney et al., 2011), the reason for this is unclear, with some research pointing towards inadequate nutrition knowledge contributing to poor food choices. Current nutrition guidelines are tailored around the physiological and training demands of the sport (Thomas et al., 2016). With enhancements in genomic and metabolic research the concept of personalised nutrition has rapidly progressed (Joost et al., 2007). The metabolic typing diet prescribes a diet individualised to the persons needs based on the output of a 65 question questionnaire (Walcott and Fahey, 2000), which claims to reset metabolic homeostasis, providing an optimum nutrition solution.

However with no research the application of the metabolic typing questionnaire as a tool to define metabolic type and dietary intake the area needs further exploration.

Optimising or enhancing the adaptation attained from a training session may lead to subsequent improvement in exercise performance. Manipulating carbohydrate availability is one such mechanism that has received a significant body of research in recent years. Mitochondrial biogenesis is a desired metabolic training adaptation from endurance exercise; this process is controlled by the AMPK/SIRT1 – PGC-1α axis. Energy restriction, carbohydrate restriction and acute exercise are known to increase the expression of AMPK/SIRT1 – PGC-1α axis; promoting mitochondrial biogenesis. The exact method and the extent of manipulation of the external stressors are yet to be fully described. The majority of human studies in this area manipulate fat to restrict carbohydrate availability; the effect of increased protein intake on metabolic adaptation of the AMPK/SIRT1 – PGC-1α axis at rest and in combination with endurance exercise is yet to be fully explored. With high protein diets currently *en vogue* and numerous reports stating that athletes are consuming high protein diets, further research into this area is needed to help provide informed decisions.

2.5.1 Experimental questions raised from literature review

This review of literature has identified a number of areas that require further study and consequently some of these will be addressed in the subsequent experimental Chapters of the thesis by raising the following questions and hypotheses:

1. Can a commercially available metabolic typing questionnaire prescribe a diet personalised to individual metabolic needs, and does an association exist between metabolic type and sporting discipline?
    - Hypothesis – The application of the metabolic typing questionnaire will identify endurance athletes as having a different metabolic type to speed and power athletes.

2. What is the level of nutrition knowledge in UK Track and Field athletes, and does a relationship exist between nutrition knowledge and dietary intake?

- Hypothesis – UK track and field athletes will have better nutrition knowledge relative to a non-athletic group, also individuals with greater nutrition knowledge will have better dietary intake.

3. What is the impact of increasing dietary protein intake independently or concurrently in a hypocaloric diet on metabolic adaptation, body composition and resting metabolic rate?
    - Hypothesis – Increased protein intake in a hypocaloric diet intake will promote greater metabolic adaptation.

4. Does an isocaloric high protein diet, in combination with endurance training have a preferential impact on metabolic adaptation, substrate utilisation and exercise performance?
    - Hypothesis – A 7-day isocaloric high protein diet, in combination with endurance training, will promote metabolic adaptation, enhance substrate utilisation and improve subsequent exercise performance compared to a high carbohydrate diet.

Specifically, the above questions and hypotheses will be addressed in the subsequently experimental Chapters;

- **Chapter 4.** To develop and validate a new reliable nutrition knowledge questionnaire specific for athletes.
- **Chapter 5.** To examine the current nutrition knowledge and dietary intake of UK track and field athletes and investigate the application of the metabolic typing questionnaire as a tool for personalised nutrition prescription.
- **Chapter 6.** Examine the impact of acute energy restriction and increased protein intake for 7-days on metabolic adaptation, body composition and resting metabolic rate at rest.
- **Chapter 7.** Investigate the impact 7-days endurance training in combination with increased protein intake has on metabolic adaptation, substrate utilisation and exercise performance.

3 GENERAL METHODS

This thesis presents data from four different studies. This Chapter describes common or complex methodological procedures used throughout the course of the studies and acts as a reference point for experimental Chapters presented later in this thesis.

3.1 Dietary Intake Analysis

The method: Dietary analysis is a method by which current habitual dietary intake can be attained and measured. Subsequent to a review of the literature (refer to Chapter 2.4.1 for a critical analysis) the estimated method using household measures diet log was selected as the tool to record and measure habitual dietary intake. The review of literature concluded that 3–4 day's estimated diet record is optimal for the research design throughout this thesis.

Table 3.1.1 .Food diary example provided to the participant.

Time	**Food item** (Please include as much detail as possible, BRAND NAME, PORTION SIZE, FOOD WEIGHT, QUANTITY, COOKING METHOD, NUTRITIONAL INFORMATION. THE MORE INFORMATION THE BETTER!	**Drink Item**
7.30 am	**Porridge:** 250 g Tesco own brand porridge with 100 ml skimmed milk and 1 teaspoon of honey (cooked in microwave). 1 large apple (60 grams). 250 ml orange juice (Tropicana with bits)	Coffee: 1 Teaspoon of Nescafe, 250 ml water, 1 teaspoon granulates sugar, 20 ml milk.
9 am	1 tablespoon of sunflower seeds, 8 cashew nuts, 150g Muller light strawberry yogurt.	500 ml San Pellegrino Sparkling Water
11 am	75 g dried Tesco Couscous with 150 ml boiling water, 1 tin of Tesco's (120 g) Sardines in tomato sauce.	1 pint water + 1 can of diet coke.
3pm	27g bag Walkers cheese and onion crisps. 227 g Ginsters Original Cornish Pasty.	Cup of tea (Tetley tea, 300 ml water, 10 ml skimmed milk)
4pm	2 slices of toast (Hovis, best of both medium), scraping of butter and teaspoon of jam on each.	500 ml Semi-skimmed milk
9pm	**Spaghetti:** 100 g dried Tesco value spaghetti (boiled for 15 minutes) (I ate all the spaghetti) **Bolognaise**: (all simmered for 30 minutes) 400g tin tomatoes, 4 button mushrooms, ½ carrot, 200 g Sainsbury's extra lean minced beef, 1 OXO stock cube, 20 ml tomato puree, 1 small onion (50 g). 2 Basil leaves. ½ tea spoon Cayenne pepper. 50 g Cathedral Cheddar Cheese. I ate half of the bolognaise.	1 pint of water
Supplements 500 mg Vitamin C (Boots). Seven Seas Omega 3 tablet. 5 g Creatine (MyProtein.com)		

The protocol: Dietary intake analysis was completed in experimental Chapters 5, 6 and 7. Prior to the start of each study the participants were provided with a comprehensive example of the completed food diary (Table 3.1.1). The importance of accuracy and detail were emphasised, as was the importance of maintaining current dietary habits and documenting all food and drink consumed. Participants were

instructed to fill in the diet log throughout the day if possible and not leave till the evening/next day. Participants were requested to record intake on two (Experimental Chapter 5) and three (Experimental Chapter 6 and 7) week-days and one weekend day during the specified week.

3.2 Food diary analysis

The method: Food diaries were inputted and analysed using a software package to quantify dietary intake.

Reliability: For each phase of study the food diaries were analysed by the same investigator. Due to a change in institution and licensing, the food diaries in Chapter 5 were analysed using Dietplan 6 (Forestfield Software Ltd, Horsham, UK) and in Chapters 6 and 7 the food diaries were analysed using Nutritics (Nutritics LTD, Dublin, Ireland). Both Dietplan6 and Nutritics are currently based on the same McCance and Widdowson (2015) (6th edition) food and nutrient composition tables as published by Public Health England, however in Chapter 5, the analytical software used, Dietplan6 was based on the 5th Edition of the McCance and Widdowson composition tables.

The protocol: Food and drink items were manually entered in a maximum of five separate meal times: breakfast, snack, lunch, snack, and dinner. If the logged food item was not present in the platform database and the participant did not provide the macronutrient breakdown of the food item, the details of the food were searched for on the internet and manually inputted into the database. As much attainable detail about the item was entered, including all macro- and micro-nutrients. If the participant, or other participants, consumed this food item at another time point the newly inputted food item was selected.

3.3 Food provision

The method: For Chapters 6 and 7 the participants were provided with a diet for the duration of the intervention. The macronutrient ratio's were set by the investigative team and provided as a brief to Soulmatefood® (Waterfoot, Lancashire) who created

the diets specific to the participant's requirements. The food packages were delivered directly to the participant's door.

Reliability: Due to the nature of diet creation and cooking, it is not possible to create a diet matching the exact prescribed calorie intake. Due to this, a total of 34 (17 high carbohydrate, 17 high protein) different diet menus were created starting at 1,075 kcal. The bandwidth of each diet was 149 calories, and each diet was created to match the median value of the band (Table 3.3.1), the largest diet provided 3,325 kcal. The greatest possible difference between estimated intervention intake and prescribed diet was 75 kcal•day^{-1}. Participants were assigned the diet closest to their calculated interventional energy intake.

Table 3.3.1. Table used to calculate the participants daily energy intake for the intervention diet.

	Calculated energy intake range (kcal•day^{-1})		**Energy provided within intervention diet (kcal•day^{-1})**
	Minimum	**Maximum**	
Diet A	1,000	1,149	1,075
Diet B	1,150	1,299	1,225
Diet C	1,300	1,449	1,375
Diet D	1,450	1,599	1,525
Diet F	1,600	1,749	1,675
Diet G	1,750	1,899	1,825
Diet H	1,900	2,049	1,975
Diet I	2,050	2,199	2,125
Diet J	2,200	2,349	2,275
Diet K	2,350	2,499	2,425
Diet L	2,500	2,649	2,575
Diet M	2,650	2,799	2,725
Diet N	2,800	2,949	2,875
Diet O	2,950	3,099	3,025
Diet P	3,100	3,249	3,175
Diet Q	3,250	3,399	3,325

The protocol: Each participant received two deliveries; the first delivery contained three days food, the second four days food. Each day consisted of 5 pre packaged / cooked meals (breakfast, morning snack, lunch, afternoon snack and dinner). Included

with the delivery was a daily menu with consumption instructions. The participants were required to refrain from consuming any additional foods and requested just to drink water or drinks free from caffeine or energy.

3.4 Micro muscle biopsy sampling

The micro muscle biopsy method (Hayot et al., 2005) was used in Chapter 6 and 7 to collect muscle tissue from the vastus lateralis muscle.

3.4.1 Introducing the technique at the University of Hertfordshire and the GSK Human Performance Lab

Prior to initiation of this thesis the method of taking muscle biopsies had not been undertaken at the University of Hertfordshire. In order to investigate the desired research questions it was essential to introduce this technique into the institution. Due to the title and invasive nature of the procedure, caution, diligence and a thorough understanding of the technique were required to ensure the university regulatory body allowed the technique to be introduced. From deciding muscle biopsies were essential for the body of research, to taking the first pilot muscle biopsy at the University of Hertfordshire, the process paperwork and training took approximately 12 months. Substantial time was taken to develop the operating procedures and organise relevant training. Refer to Appendix Two for the detailed standard operating procedure (SOP) created to introduce the technique. In brief, the steps taken to introduce the technique at the University of Hertfordshire were as follows:

1. Scoped technique and detail out stages needed for completion prior implementation.
2. Discussed with the technical team.
3. Engaged with external expert to observe the technique.
4. Presented technique and method to the health and safety lead along with ethics committee.
5. Completed external training.
6. Gained ethics approval for the technique.
7. Completed pilot biopsies.

Subsequent to introducing the technique at the University of Hertfordshire, a change in employment occurred and similar processes had to be undertaken to introduce the technique to a new laboratory (GSK Human Performance Lab).

3.4.2 The micro muscle-biopsy technique

The reclining biopsy couch was cleaned with a sterilising solution; the participant then sat or lay (dependent on comfort) on the couch. Muscle biopsies were obtained from the approximated midpoint on the lateral aspect of the right vastus lateralis muscle. In order to select the target biopsy site the participant was requested to contract the selected quadriceps muscle group to define the vastus lateralis muscle, the selected site was subsequently marked. A small amount of Betadine (Pardue Products, USA) was poured on to sterile gauze and used to clean the biopsy site and surrounding area (~15 x 15 cm^2). Biopsy samples were obtained under local anaesthesia; 2 mL of 1 % v/v without adrenaline Lidocaine Hydrochloride (Hameln Pharmaceuticals: cat. no PL01502) was injected into the subcutaneous fat of the selected biopsy site. Once the anaesthetic had taken effect (~5 minutes) a 14 gauge co-axial was inserted, at an ~30° angle to the leg, through the skin and subcutaneous fat and ~2 cm into the muscle of the biopsy site. The insertion needle of the co-axial was removed leaving the co-axial in the leg. A disposable core biopsy instrument (TSK Stericut Biopsy Needle 14 Gauge, TSK Laboratories, Japan) was inserted through the co-axial and activated. A single pass was used collecting approximately 10 – 20mg of muscle tissue. The core biopsy instrument was immediately removed from the leg, and within ~10 seconds the muscle tissue was removed from the biopsy instrument using a sterile scalpel (disposable scalpel number 11, Swann-Morton, UK) and placed in liquid nitrogen to be flash frozen. The muscle samples were stored in a -80 ^oC freezer under the HTA license number 12202 until analysis. On subsequent visits, the repeat biopsies were taken approximately 10 mm away laterally from the original biopsy site along the vastus lateralis muscle (Figure 3.4.1).

Figure 3.4.1. Example image of sites selected for the three biopsies.

The same site selection process was used for both Chapters involving the technique.

3.5 mRNA extraction and analysis

Quantitative Polymerase chain reaction (qPCR) was selected as the method for quantification of mRNA expression instead of traditional PCR for a number of justified reasons. Firstly qPCR measures the amplification as it occurs, whereas traditional PCR only measures the accumulated PCR produce at the end of the PCR cycles. As such, qPCR is quantitative because data is collected during the exponential growth phase of PCR when the quantity of the PCR product is directly proportional to the amount of template nucleic acid; traditional PCR provided semi quantification through the intensity of the amplified band. Furthermore qPCR allows for quantification of gene expression and is more sensitive than traditional PCR.

3.5.1 RNA Isolation

Human muscle biopsies were homogenised in 700 µL MagNA Pure LC RNA Isolation Tissue Lysis buffer (Roche, Mannheim, Germany) in Roche MagNA Lyser Green bead tubes at 6500 rpm for 30 seconds. After homogenisation, the tubes were centrifuged for 10 minutes at 13,000 g before 350 µL of each homogenate was used for RNA extraction. Total RNA was extracted using the MagNA Pure 96 Cellular RNA Large Volume Kit on a MagNA Pure 96 (Roche, Mannheim, Germany), in an elution volume

of 50 µL according to the manufacturer's protocol. RNA was stored at -80ºC. RNA concentrations were determined (A260) using a NanoQuant plate on a Tecan Infinite 200PRO. RNA quality (RNA integrity number equivalent, RINe) was assessed using RNA ScreenTape on a 2200 TapeStation (Agilent, Santa Clara, USA).

3.5.2 Reverse Transcription

As a result of the large sample number and the automated process, RNA concentration was not taken into account for each individual reaction. 14 µL of each RNA sample was reverse transcribed in a total volume of 20 µL using the High capacity cDNA reverse transcription kit (without RNase inhibitor, Applied Biosystems, Thermo Fisher, Loughborough, UK). Reactions were performed in 96 well PCR plates on a PTC-225 Peltier thermal cycler (MJ Research, Quebec, Canada) using the following profile: 25°C for 10 minutes, 37°C for 60 minutes, 85°C for 5 minutes, 4°C hold. Minus RT control reactions were set up for 14 samples, in which the RNA component was replaced with nuclease free water (Ambion, Fisher Scientific, Loughborough, UK).

3.5.3 qPCR

For quantitative real-time PCR (qPCR), Human TaqMan® gene expression assays were purchased as a 20X assay ready stock from Life Technologies (Carlsbad, USA) (primers 18mM and probes mM) (Table 3.5.1). 1 µL cDNA was added to each qPCR reaction mixture which also contained gene expression assay mix (primers 900 nM final, probe 250 nM final), LightCycler 480 Probes Master and nuclease free water to give a 5µL total reaction volume. Reactions were prepared in white multiwell 384 plates (Roche, Mannheim, German) using a Mosquito HV (TTP Labtech, Melbourn, UK). The plates were sealed using optical seals (Roche, Mannheim, Germany) and centrifuged at 290 g for two minutes before being run on a LightCycler® 480 instrument (Roche, Mannheim, Germany) with the following thermal cycling parameters: initial de-naturation step 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 10 seconds and primer annealing/extension at 60°C for 30 seconds. A cooling step at 40°C for 30 seconds was the final stage of the run.

Table 3.5.1. qPCR gene code and catalogue number

Gene name	Gene abbreviation	Code
Silent information regulator-T1 (SIRT1)	SIRT1	Hs01009005_m1
Silent information regulator-T3 (SIRT3)	SIRT3	Hs00953477_m1
AMP-activated protein kinase 1 (AMPK)	PRKAA1	Hs01562315_m1
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)	PPARGC1	Hs01016719_m1
Peroxisome proliferator-activated receptor delta (PPAR)	PPARD	Hs00987011_m1
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	GAPDH	Hs99999905_m1

The crossing point (Cp) value for each sample was calculated using the second derivative maximum method applied directly by the Roche software to the real-time amplification curves. This value represents the cycle at which the increase of fluorescence is highest and where the logarithmic phase of a PCR begins.

3.5.4 Amplification Efficiency of qPCR assays

The efficiency of each of the Taqman® assays on demand was confirmed by performing standard curves in a 384 well qPCR assay on the LightCycler® 480 under the same conditions as already described (Section 3.5.3). Six point standard curves with 1 in 10 serial dilutions were prepared in nuclease free water for human plasmid DNA for each of the genes being tested alongside Human genomic DNA with a top concentration of 3e5 copies/µL and 1e5 copies/µL respectively. qPCR reactions were performed in triplicate and the amplification efficiency calculated for each Taqman® assay on the basis of the equation $E = (10^{(-1/slope)} - 1) \times 100$ with the logarithm of the template concentration on the x axis and the average Cp plotted on the y axis.

3.6 Dual energy X-ray absorptiometry (DXA) scan.

The method: The DXA scan was used in experimental Chapters 6 and 7 to quantify lean mass, fat free mass and body mass. It is described as a gold standard method of body composition assessment (Fowke and Matthews, 2010).

Quality assurance (QA) of the DXA scan (GE Lunar iDXA, GE Healthcare, UK) was completed first thing in the morning every day throughout the data collection in experimental Chapter 6 and 7 in accordance with the manufacture guidelines. QA mode was selected on the enCORE software (version 14.10, GE Healthcare, Bucks, UK). The calibration block was placed in position and lined up with the laser. The QA was then started. The QA passed every time with a coefficient of variation (CV) for this QA procedure of 0.07%.

Procedure: All metal and piercings were removed and fasted body mass was measured using a digital column scale (Seca 704, Seca Ltd., Hamburg, Germany). The participant was then positioned in the centre of the scanner table, using the centreline on the table as a reference to align the patient. The participant's hands were turned on the side with thumbs pointing up and palms facing the body. Hands were positioned as not to touch the legs and a small air gap (~1 cm) between the arms and torso was ensured. The ankles were positioned ~20 cm apart, with a Velcro strap placed around to support the legs and reduce movement. After verification that the participant was positioned within the scan area the participant was requested to remain still throughout the scan. For all participants the scan settings on the enCORE software (version 14.10, GE Healthcare, Bucks, UK) was set to standard thickness and a full body DXA scan (GE Lunar iDXA, GE Healthcare, UK) was performed. The scan took approximately 7 minutes 16 seconds to complete. A fully trained operator completed all scans.

Radiation is produced from the DXA scanner when electric voltage is supplied to, and current flows through, the x-ray tube. During a measurement, the shutter opens to let a beam of radiation pass through the scanner table and patient. Estimated skin entrance dose from a single full body scan for under the standard thickness setting is 3 μGy (GE Lunar iDXA, GE Healthcare, UK), each participant received a maximum radiation dosage of 9 μGy.

3.7 Assessment of expired air using an online gas analyser.

The method: An online (i.e. continuously measured/real time) gas analyser (Metalyzer 3B, Cortex, Leipzig, Germany) was used in experimental Chapter 7 to measure oxygen and carbon dioxide fractions, and volume of gas in inspired and expired air.

Reliability and calibration of the online gas analyser: The reliability data on the Metalyzer was completed prior to the trial started; three participants completed the same exercise protocol for three consecutive days at the same time of day. Using a cycling protocol participant rode at 50, 100, 150, 200, 250 and 300 watts for 4 minutes. The last 30 seconds of data collection for each stage was averaged and used to calculate reliability.

Table 3.7.1. The coefficient of variation (CV) of the online gas analyser at different power outputs and $\dot{V}O_2$.

Power (W) Absolute	**$\dot{V}O_2$ ($L \cdot min^{-1}$)**	**Mean Coefficient of Variation (%)**			
		HR	**$\dot{V}O_2$**	**$\dot{V}CO_2$**	**RER**
50	1.16	6.02	5.49	8.13	3.17
100	1.66	3.11	2.22	4.33	2.70
150	2.13	2.98	2.41	2.17	1.36
200	2.71	1.20	2.54	2.25	1.02
250	3.26	0.98	3.20	3.37	1.34
300	3.91	1.05	1.63	1.96	1.05

Procedure: The analyser was warmed-up for a minimum of 45 minutes and calibrated for oxygen (17 %) and carbon dioxide (5 %) fractions and gas volume (3 L syringe, Hans Rudolph) as per manufacturer's prescription. During the tests, the participant breathed through a low dead space (70 mL) mouthpiece, low resistance turbine (<0.1 $kPA \cdot L^{-1} \cdot s^{-1}$ at 16 $L \cdot s^{-1}$), whilst inspired and expired gas was sampled continuously at 50 Hz. The analyser rise time and transit delay for O_2 and CO_2 were <100 ms and 800-1200 ms respectively, using a dynamic calculation for each breath.

3.8 Run testing using a treadmill.

The method: In Chapter 7 a protocol was designed using a treadmill to measure (i) substrate utilisation during a sub maximal run and (ii) a maximal sustainable effort run. As described in Chapter 7 the participant was directed to run on the treadmill at a set speed. All experimental testing in experimental Chapter 7 was completed on the same treadmill (Cosmed T170, Rome, Italy).

Reliability and calibration: Treadmill reliability and calibration were completed prior to the study loaded and unloaded. The length of the treadmill belt was measured and a mark was drawn on the belt and foot stand. The selected calibration speed was set on the treadmill controller and once the belt was up to speed a stopwatch was started and belt revolutions were counted every time the markers crossed each other using a tally counter. Once 100 revolutions were completed the stopwatch was stopped and time recorded. This was repeated twice (Table 3.8.1 and Figure 3.8.1 and Table 3.8.2 and Figure 3.8.2).

Table 3.8.1. Reliability and accuracy of the treadmill unloaded.

		Time 100 belt revolutions (seconds)			**Actual Speed**	
	Speed ($km \cdot h^{-1}$)	**Trial 1**	**Trial 2**	**Mean**	**($m \cdot s^{-1}$)**	**($km \cdot h^{-1}$)**
26/01/2016 No load 1 % gradient 4.155 m belt length	5	296.6	297.36	296.98	1.40	5.04
	10	147.79	147.78	147.79	2.81	10.12
	15	99.42	98.14	98.78	4.21	15.14
	20	74	74.12	74.06	5.61	20.19
	25	59.97	58.97	59.47	6.98	25.15

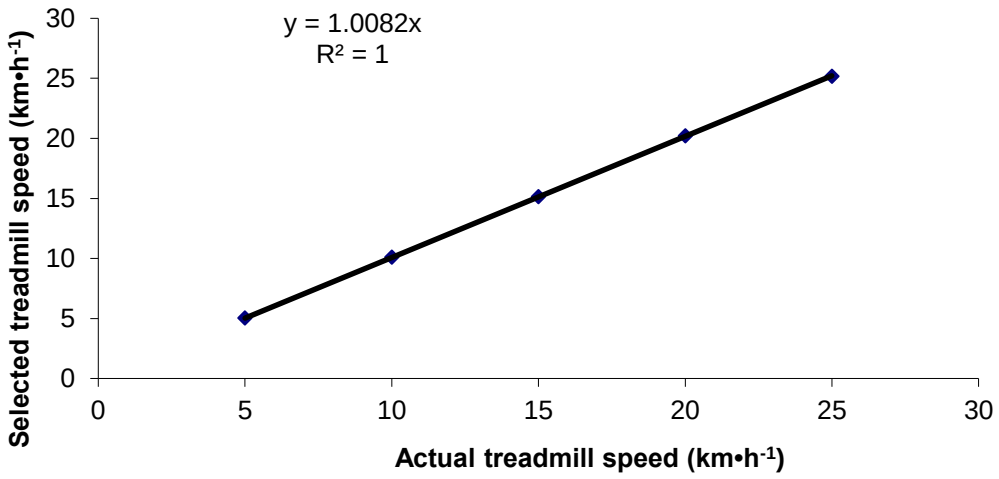


Figure 3.8.1. Correlation between the selected treadmill belt speed and unloaded calculated actual speed.

Table 3.8.2. Reliability and accuracy of the treadmill loaded with participant running.

		Time 100 belt revolutions (seconds)			**Actual Speed**	
	Speed ($km \cdot h^{-1}$)	**Trial 1**	**Trial 2**	**Mean**	**($m \cdot s^{-1}$)**	**($km \cdot h^{-1}$)**
26/01/2016 91 kg load 1 % gradient 4.155 m belt length	5	296.78	296.74	296.76	1.40	5.04
	10	148.49	148.48	148.49	2.80	10.07
	15	98.52	98.60	98.56	4.22	15.18
	20	74.03	73.81	73.92	5.62	20.24

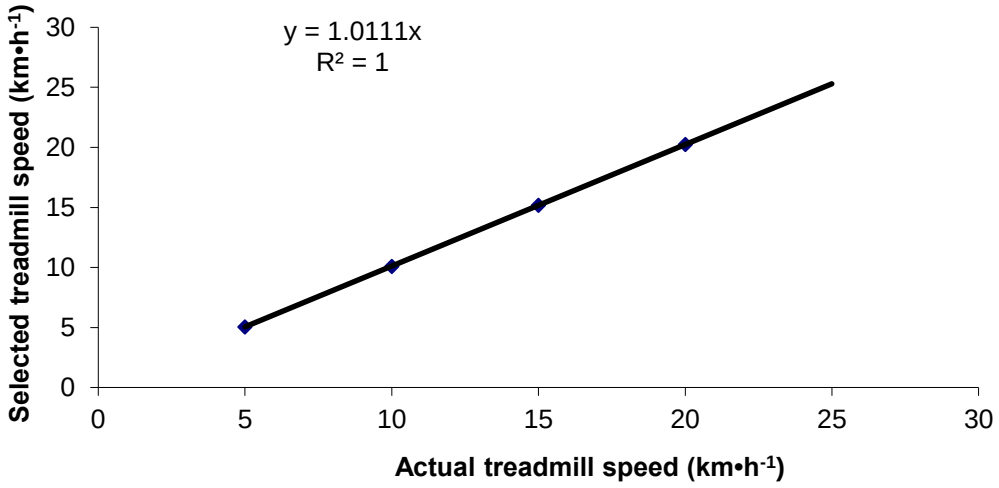


Figure 3.8.2. Correlation between the selected treadmill belt speed and loaded calculated actual speed.

3.9 Measuring blood glucose and blood lactate

The method: The EKF Biosen C_line (EFK Diagnostics, Barleben, Germany) was used to measure blood glucose and lactate in Chapter 7.

Calibration and Reliability: Monthly reliability checks were carried out on the EKF Biosen using glucose/lactate linearity check solution (02090102391A, EKF Biosen, Barleben, Germany) with three concentrations 2, 7 and 12 mmol•L^{-1}. Two separate vials of each concentration were fun five times for reliability (Table 3.9.1).

Table 3.9.1. Mean, standard deviation (SD) and coefficient of variation (CV) of the EKF Biosen throughout the data collection phase of experimental Chapter 7.

Date	Concentration (mmol•L^{-1})	Reliability					
		Mean		SD		CV (%)	
		# 1	# 2	# 1	# 2	# 1	# 2
01/02/2016	2	1.97	1.93	0.01	0.02	0.45	0.87
	7	6.94	7.03	0.01	0.02	0.10	0.30
	18	17.62	18.25	0.02	0.05	0.11	0.26
22/03/2016	2	1.95	1.94	0.01	0.00	0.43	0.23
	7	6.95	7.04	0.01	0.01	0.08	0.10
	18	17.82	18.27	0.01	0.01	0.06	0.03
05/04/2016	2	1.97	1.96	0.02	0.01	0.83	0.56
	7	7.15	7.11	0.01	0.02	0.17	0.21
	18	18.33	18.50	0.02	0.03	0.10	0.15

The protocol: The selected finger was cleaned with an alcohol wipe and left to air dry. The finger was then punctured with a capillary lancet, the first drop of blood was wiped away and a sample of whole capillary blood was collected using a 20 µL capillary tube. The capillary tube was checked to ensure no air bubbles were present. The capillary tube was placed in a plastic vial containing 1 mL of haemolysing solution (Gluc/Lact Haem Solution, EKF Diagnostics, Barleben, Germany) and shaken ten times to ensure all the blood had exited the capillary tube and mixed evenly with the haemolysing solution. The vial was then placed into the EKF Biosen and analysed for glucose and lactate concentrations.

Analysis: The EKF Biosen uses an electrochemical dual-channel chip sensor to analyse blood glucose and lactate concentrations. Blood lactate and glucose are converted enzymatically with glucose oxidase and lactate oxidase producing H_2O_2 which is detected at the electrode and the current is proportional to the analyte concentration.

3.10 Indirect calorimetry using the GEM Nutrition open circuit indirect calorimeter.

The Method: The GEM is an open circuit indirect calorimeter designed to measure energy balance and substrate turnover.

Calibration: The GEM was turned on and warmed up for a minimum of 30 minutes prior to calibration. The air flow through the hood was set to 50 litres•min-1 Using a span gas of 1% CO2 and 21% O2 along with a zero gas of 99.998% N2 the GEM was calibration according to manufactures guidelines. The GEM was calibrated prior to every test.

4 DEVELOPMENT OF A VALID AND RELIABLE NUTRITION KNOWLEDGE QUESTIONNAIRE FOR ATHLETES.

Abstract

Background*:* Establishing an understanding of an athlete's nutrition knowledge can inform the coach/practitioner and support the development of the athlete. Thus the purpose of the study was to develop a psychometrically valid and reliable tool to assess general and sport nutrition knowledge. ***Methods*****:** An 85 question questionnaire was developed in consultation with a panel of experts. Ninety-eight participants from the UK completed the questionnaire, and again three weeks later. The participants were classified into two groups: those with nutrition (NUT, n = 53) training (sport nutritionists and dietitians who were either practicing or undertaking a postgraduate qualification in the field), and those without (NONUT, n = 48) training (professionals and postgraduate students with no exposure to any form of nutrition training). ***Results****:* Psychometric statistical analysis of the results was completed, resulting in the removal of 23 questions for a total of 62 questions in the final questionnaire. The validated questionnaire was then administered to 58 track and field athletes. Internal consistency was assessed using Chronbach's alpha ($\alpha > 0.7$), Pearson's correlation ($p < 0.05$) was used to assess reliability. Construct validity was evaluated using a *t*-test ($p < 0.05$). A total test retest correlation of 0.95 was achieved (sub-section range: 0.87–0.97). Internal consistency was accepted in each sub-section ($\alpha = 0.78–0.92$) and the nutrition-trained group scored significantly higher on the overall questionnaire (80.4% vs 49.6%). ***Conclusion****:* The questionnaire satisfied all psychometric measures and provides a new valid and reliable tool to assess general and sport nutrition knowledge.

4.1 INTRODUCTION

Nutrition plays an important role in human health, it is postulated that nutrition is the most controllable risk factor impacting long-term health and chronic disease (World-Health-Organization, 2003) and can be easily manipulated to improve exercise performance (Burke and Deakin, 2010). Consequently, optimal health and sport nutrition strategies have been subject to comprehensive research (Magkos and Yannakoulia, 2003). However recommendations may be controversial and can be misinterpreted, as such the sport and fitness industries are saturated with varying opinion, articles and internet material which can provide unsubstantiated claims (Smith-Rockwell et al., 2001). Furthermore athletes' diets are commonly reported as being nutritionally inadequate (Heaney et al., 2011). As described in Chapter 2.1.6 the underlying reasons for this are unclear. Developing a valid and reliable tool to assess knowledge with the potential of ruling out a knowledge issue, or having grounds for an intervention to address inadequate knowledge, would prove valuable.

To develop a valid and reliable instrument to measure psychological attributes a defined set of criteria needs to be met (Kline, 2007b),a predefined structure should be followed and a series of measures must be performed (Kline, 2007b) (Refer to Table 2.2.1 in Chapter 2.2.1). A number of nutrition knowledge questionnaires have previously been developed using a range validation methods, targeting specific populations; New Zealand rugby coaches (Zinn et al., 2005), South African adolescents (Whati et al., 2005), elderly (Thomas et al., 1990) and inpatients (Anderson et al., 2002), however the validity of the instrument is reduced if used in different populations.

Designing a valid and reliable tool to assess general and sport nutrition knowledge in an athletic population may provide the accurate information needed to advise better dietary choices and improve dietary intake (Spendlove et al., 2012). Currently, to the authors' knowledge, only two validated and reliable questionnaires exist which assess sport nutrition knowledge. Rosenbloom et al. (2002) developed an 11-question tool for the assessment of nutrition knowledge in division 1 collegiate athletes, however all questions were performance nutrition related. Zinn et al. (2005) developed a psychometrically valid and reliable sports nutrition knowledge questionnaire designed

for the application on New Zealand rugby union coaches, which lacked a comprehensive general nutrition knowledge section. There is a clear need for a psychometrically validated nutrition knowledge measure that can investigate both general and sport nutrition knowledge; the aims of this research were to develop a valid and reliable general and sport nutrition knowledge questionnaire.

4.1.1 Experimental hypotheses

i) (**H_1**) The steps taken to develop the questionnaire will result in a valid and reliable questionnaire.
ii) (**H_0**) The steps taken to develop the questionnaire will not result in a valid and reliable questionnaire.

4.2 METHODS

4.2.1 Study Design

The project was approved by the University of Hertfordshire Life and Medical Sciences ethics committee (protocol number LS/4/5/11P) and was designed to develop a new tool for measuring nutrition knowledge in athletes. Eight separate processes were used to generate the final version of the Nutrition Knowledge Questionnaire for Athletes (NKQA), detailed in Figure 4.2.1. Following a comprehensive literature search the topics and subsections of the questionnaire were developed. Each question was discussed in an expert review meeting and critiqued for comprehension, detail and relevance and was either ‘removed’, ‘changed’, or ‘left in’. The questionnaire was uploaded online and administered to the participants twice in a test retest manner, separated by a three-week period. This period was considered long enough for the answers to be forgotten, but short enough to minimise any real change in nutrition knowledge (Kline, 2007b). Participants were asked to record and note the time it took to complete.

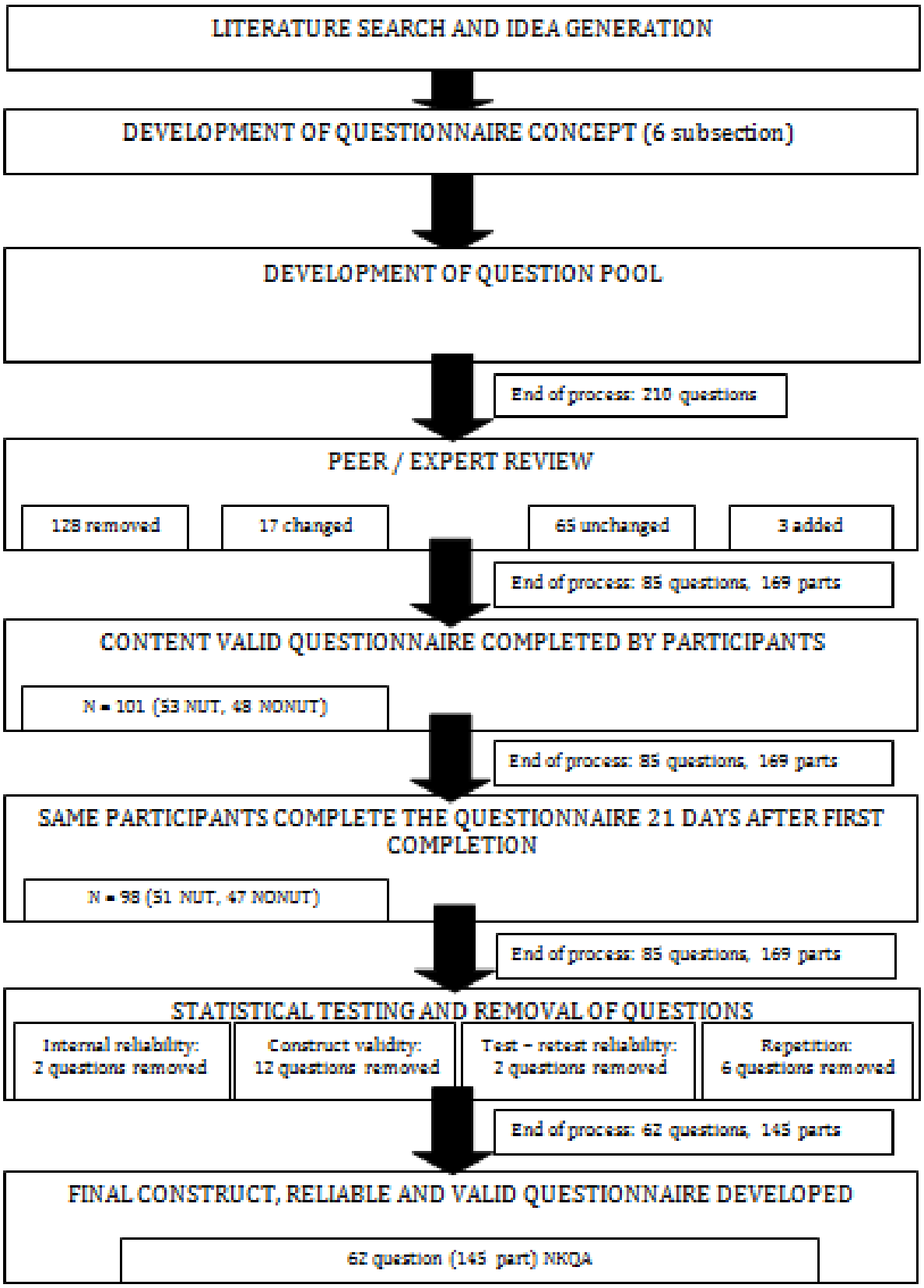


Figure 4.2.1. Schematic of processes completed to develop the NKQA.

At the time of the initial administration the participants were not made aware of the second administration. The results from the first collection were used to assess internal consistency and construct validity; the data from both collection phases was used to assess reliability over time.

4.2.2 Participants

Two population groups with differing exposure to nutritional training were selected to receive the questionnaire. The two groups were matched in education level, but with different professional expertise. The nutrition group (NUT) (*n* = 53) consisted of sport nutritionists and dietitians who were either practicing or undertaking a postgraduate qualification in the field. The non-nutrition group (NONUT) (*n* = 48) consisted of a range of professionals and postgraduate students with no exposure to any form of nutrition training (Table 4.2.1). Participants were recruited via the use of an email flyer and voluntarily contacted the research team to partake in the study.

Table 4.2.1. Participant characteristics.

Characteristics (sex)	1st collection (*N* = 101)				2nd collection (*N* = 98)			
	NUT		NONUT		NUT		NONUT	
	n	%	n	%	n	%	n	%
Male	31	64	26	54	30	59	26	55
Female	22	36	22	46	21	41	21	46

NUT = nutrition trained group, NONUT = no nutrition training group.

4.2.3 Assessment of validity, reliability and statistical analysis.

4.2.3.1 Content validity of the NKQA:

The American College of Sports Medicine (American Dietetic et al., 2009) position stand is an academic peer review synopsis on current literature and recommendations in the field of sport nutrition and good nutrition practices for athletes, thus it was adopted as the reference to develop the answer structure. The questionnaire was developed from a pool of 210 questions. The questions were either adapted from previous questionnaires (Zinn et al., 2005, Parmenter and Wardle, 1999) or contrived from the use of literature searches and expert opinion. It was decided to have six

definitive subsections within the questionnaire; carbohydrate, protein, fat, general nutrition, fluid and sports nutrition. These six areas were deemed important to assess as each impact either health, sporting performance or both. A meeting was held with 5 experts in the field (2 x graduate nutritionists – both with > 3 years experience working in elite sport, 2 x nutritionists – both Senior Lecturers, 1 x physiologist – Principal Lecturer) to discuss the merits of the questionnaire. Each question was rigorously critiqued for its comprehension, relevance, accuracy and scientific support and was either 'removed', 'changed', or 'left in'. Consequentially 128 questions were removed, 17 had wording changes, 3 were added and 65 remained un-changed, resulting in an 85-question questionnaire. A number of questions included multiple parts, for example: Are the following foods high or low in carbohydrate: Beef, Lentils or Jelly babies? In total the 85 questions contained 145 parts, taking approximately 15-20 minutes to complete.

4.2.3.2 Construct validity of the NKQA:

To demonstrate that the questionnaire differentiated nutrition knowledge the construct validity of the questionnaire was assessed. An independent sample *t*-test was performed comparing the mean subsection and total scores achieved on the questionnaire from both the NUT and NONUT groups. A significance of $p \leq 0.05$ was used.

4.2.3.3 Test re-test of the NKQA:

A test-retest measurement was used to assess reliability over time. A Pearson's Correlation was used to assess the correlation between the results from 98 participants who took the questionnaire at the two separate time points; a significance of $p \leq 0.05$ was selected.

4.2.3.4 Internal consistency of the NKQA:

Each of the six subsections were assessed separately for internal consistency as each subsection addressed a different area of knowledge. A Chronbach's Alpha, with a minimum requirement of $\alpha > 0.7$ was accepted to demonstrate sound internal

consistency (Kline, 2007b). The Chronbach's Alpha statistical test is commonly used to measure and assess for internal consistency in questionnaires.

General and Sport Nutrition Knowledge Questionnaire

Fill out the questions below to the best of your knowledge. Please **do not guess**, if you are unsure of an answer please tick the '**UNSURE**' box.

CARBOHYDRATE

1. In general are these foods **High** or **Low** in carbohydrate? *(Please tick one box per food).*

	High	Low	Unsure
Beef	☐	☐	☐
Pasta	☐	☐	☐
Cabbage	☐	☐	☐
Weetabix	☐	☐	☐
Chocolate spread	☐	☐	☐
Lentils	☐	☐	☐
Wholemeal bread	☐	☐	☐
Chicken	☐	☐	☐
Jelly babies	☐	☐	☐

2. In general as an athlete, what percentage of your diet should be made up from carbohydrate? *(Please tick one box)*

10 - 25 % ☐
25 - 40 % ☐
50 - 65 % ☐
65 - 80 % ☐
Unsure ☐

3. Do you know what the glycemic index is?

Yes ☐ No ☐

If you answer no, please go to question 7.

4. Which of the following phrases best describes the glycemic index? *(Please tick one box).*

The amount of carbohydrate a food contains ☐
The extent to which carbohydrate food raises blood sugar levels ☐
The extent to which protein food raises blood sugar levels ☐
The extent to which carbohydrate food raises blood pressure ☐
Unsure ☐

5. In general is it best to eat a diet rich in **High** or **Low** glycemic index carbohydrates *(Please tick one box).*

High	Low	Unsure
☐	☐	☐

6. Which of these foods are classified as **High** or **Low** in the glycemic index? *(Please tick one box per food).*

	High	Low	Unsure
Porridge oats	☐	☐	☐
Chick Peas	☐	☐	☐
Sweets (e.g. Gummi Bears)	☐	☐	☐
Dark chocolate (>70%coca)	☐	☐	☐
Honey	☐	☐	☐
Peanut butter	☐	☐	☐
Basmati rice	☐	☐	☐
Jacket potato	☐	☐	☐

7. Brown sugar is a healthier alternative the white sugar *(Please tick one box)*

True ☐ False ☐ Unsure ☐

8. A high carbohydrate diet helps to reduce protein breakdown in the body.

Agree ☐ Disagree ☐ Unsure ☐

PROTEIN

9. Would you **agree** or **disagree** with the following statements? *(Tick one box per question).*

a) When lifting heavy weights the body uses protein as its main energy source.

Agree ☐ Disagree ☐ Unsure ☐

b) Chicken is a very good source of energy to help fuel high intensity exercise.

Agree ☐ Disagree ☐ Unsure ☐

10. Are the following foods **High** or **Low** in protein? *(Tick one box per food)*

	High	Low	Unsure
Chicken	☐	☐	☐
Kidney beans	☐	☐	☐
Potato	☐	☐	☐
Spaghetti	☐	☐	☐
Tuna	☐	☐	☐
Egg	☐	☐	☐
Peanut butter	☐	☐	☐
Spinach	☐	☐	☐
Cornflakes	☐	☐	☐
Malt Loaf	☐	☐	☐

11. What is the main use for protein in the body? *(Please tick one box).*

Energy Source ☐
Growth and repair ☐
Improve hydration ☐
All of the above ☐
Unsure ☐

12. How much protein is there in the following food items? *(please tick one box per question)*

1 pint of skimmed milk	0.6 grams	6 grams	12 grams	40 grams	Unsure
250 g tin of tuna	10 grams	20 grams	35 grams	50 grams	Unsure
1 slice of white bread	0 grams	2 grams	4 grams	8 grams	Unsure

13. In general how much protein (grams) should an average 70 kg male athlete eat per day? *(Please tick one box)*

49 – 77 grams ☐
105 – 119 grams ☐
154 – 196 grams ☐
217 – 266 grams ☐
Unsure ☐

14. There is more protein in a glass of whole milk compared to skimmed milk? *(True or false?).*

True ☐ False ☐ Unsure ☐

FAT

15. For improvements in health, what type of fat do experts recommend should be reduced in the diet? *(Please tick one box).*

Monosaturated fat ☐
Polyunsaturated fat ☐
Saturated fat ☐
Unsure ☐

16. Are these foods high in **saturated** or **poly-unsaturated** fat *(Please tick one box per food)?*

	Saturated	Poly - unsaturated	Unsure
Pumpkin Seeds	☐	☐	☐
Pork Chop	☐	☐	☐
Butter	☐	☐	☐
Olive Oil	☐	☐	☐
Mackerel	☐	☐	☐
Packet of Crisps	☐	☐	☐

17. Which of the following lipoproteins increase cholesterol? (HDL = high density lipoprotein) or (LDL = low density lipoprotein) *(Please tick one box)*

HDL's ☐
LDL's ☐
Unsure ☐

18. The following foods are high in cholesterol. *(Please tick one box per item)*

	True	False	Unsure
Coconut Oil	☐	☐	☐
Red Meat	☐	☐	☐
Eggs	☐	☐	☐
Walnuts	☐	☐	☐
Whole Milk	☐	☐	☐
Sunflower Seeds	☐	☐	☐
Sardines	☐	☐	☐

19. Do you think these foods are High or Low in fat? *(Please tick one box per item).*

	High	Low	Unsure
Orange	☐	☐	☐
Avocado	☐	☐	☐
Cottage cheese	☐	☐	☐
White bread	☐	☐	☐
Peanuts	☐	☐	☐
Sunflower seeds	☐	☐	☐
Jam	☐	☐	☐
Banana	☐	☐	☐

VITAMINS AND MINERALS

20. Do you **agree** or **disagree** with the following statements *(Please tick one box per question)*

a) 500 ml of orange juice has the same number of calories as 500ml of orange squash.

Agree ☐ Disagree ☐ Unsure ☐

b) There is more calcium in a glass of whole milk than skimmed milk.

True ☐ False ☐ Unsure ☐

21. What is the role of antioxidants in the body? *(Please tick one box)*

Help with energy production ☐
Help prevent against cell damage ☐
Increase metabolic rate ☐
Improve hydration status ☐
Unsure ☐

22. In General the following foods are naturally rich in antioxidant. *(True or false).*

	True	False	Unsure
Red Meat	☐	☐	☐
White Fish	☐	☐	☐
Fruit	☐	☐	☐
Vegetables	☐	☐	☐
Dairy	☐	☐	☐
Unsure	☐	☐	☐

23. If you want to eat something that is rich in Vitamin C, which of the following foods would you eat? *(Please tick one box per question)*

a) Oranges	☐	Beef	☐	Unsure ☐
b) Red pepper	☐	Spinach	☐	Unsure ☐
c) Whitebait	☐	Baked beans	☐	Unsure ☐

24. If you want to eat something that is rich in iron, which of the following foods would you eat? *(Please tick one box per question)*

a) Spinach	☐	Milk	☐	Unsure ☐
b) Chickpeas	☐	Banana	☐	Unsure ☐
c) All Bran	☐	Yoghurt	☐	Unsure ☐

25. If you want to eat something that is rich in calcium, which of the following foods would you eat? *(Please tick one box per question)*

a) Spinach	☐	Chicken	☐	Unsure ☐
b) Skimmed Milk	☐	Sunflower seeds	☐	Unsure ☐
c) Potato	☐	Cheddar cheese	☐	Unsure ☐

26. Which cooking method is best to help maintain the vitamin and minerals content within vegetables. *(Please tick one box).*

Steam ☐
Boil ☐
Fry ☐
Grill ☐
Unsure ☐

27. B vitamins are important for exercise because they help with: *(Please tick one box).*

Hydration status ☐
Energy Production ☐
Immunity ☐
All of the above ☐
Unsure ☐

GENERAL NUTRITION

28. Cutting out 10 grams of carbohydrate from your diet will result in greater weight loss than cutting out 10 grams of fat. *(Please tick one box).*

True ☐ False ☐ Unsure ☐

29. What food category are vegetables classified in? (Such as cabbage, carrots, onions, peppers, courgettes etc) *(Please tick one box).*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

30. During resting conditions, which is the predominant energy source the body uses? *(Please tick one box).*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

31. How many calories are there in 1 gram of each of the following macronutrients and Alcohol? *(Please tick one box)*

	Carbohydrate	Protein	Fat	Alcohol
2 kcal	☐	☐	☐	☐
4 kcal	☐	☐	☐	☐
7 kcal	☐	☐	☐	☐
9 kcal	☐	☐	☐	☐
13 kcal	☐	☐	☐	☐
Unsure	☐	☐	☐	☐

32. Does one glass of orange squash count towards the government recommended 5 a day? *(Please tick one box)*

Yes ☐
No ☐
Unsure ☐

33. If you are trying to lose weight and want to have a snack what would be best the best food to snack on? *(Please tick one box per question).*

a) Peanut butter on a bagel	☐	or	Tuna sandwich	☐
b) 100 g chicken wrap	☐	or	100 g pork pie	☐
c) 150 g Tomato salad	☐	or	100 g rice pudding	☐
d) Cottage cheese and berries (50 grams)	☐	or	Cheddar Cheese and crackers (50 grams	☐

Fluid

34. In general, how much fluid should you drink on average per day? (Please tick one box)

0 – 0.99 litres ☐
1 – 1.99 litres ☐
2 – 2.99 litres ☐
3 – 3.99 litres ☐
4 – 4.99 litres ☐
Unsure ☐

35. During exercise greater than one hour, what are the current guidelines for fluid consumption? *(Please tick one box).*

0 – 200 ml ☐
200 – 400 ml ☐
400 – 800 ml ☐
800 – 1,200 ml ☐
1,200 ml + ☐

36. In general, at what percentage of body dehydration would you start to see a decrease in exercise performance? *(Please tick one box).*

0.5 % ☐
2 % ☐
4 % ☐
6 % ☐
8 % ☐
Unsure ☐

37. A small amount of sodium added to fluid will help increase water absorption and improve hydration status? *(Please tick one box).*

True ☐ False ☐ Unsure ☐

38. In the first 2 hours after exercise how much fluid should you aim to drink?

10 % of sweat loss ☐
50 % of sweat loss ☐
75 % of sweat loss ☐
100 % of sweat loss ☐
150 % of sweat loss ☐

39. What type of drink would be best to consume in the following situations? *(Please tick one box per situation).*

	Hypotonic	Isotonic	Hypertonic	Unsure
a) Exercise lasting 0 – 45 minutes	☐	☐	☐	☐
b) Exercise lasting 45 – 90 minutes	☐	☐	☐	☐
c) Post exercise	☐	☐	☐	☐

40. How much carbohydrate is there in an isotonic sports drink? *(Please tick one box).*

0 – 3 % ☐
4 – 8 % ☐
8 – 11 % ☐
12 – 15 % ☐
Unsure ☐

41. The following drinks are isotonic. *(Please tick one box per question)*

	True	False	Unsure
a) Lucozade Sport	☐	☐	☐
b) Gatorade	☐	☐	☐
c) Red Bull	☐	☐	☐
d) Water	☐	☐	☐
e) Forgoodness Shakes	☐	☐	☐

SPORTING PERFORMANCE AND SUPPLEMENTATION
Please note, if you have not heard of any of the supplements listed below or are unsure of how they work, please tick the UNSURE box.

42. A high protein meal 1 hour before competing in a power event is recommended to enhance performance. *(Please tick one box)*

True ☐ False ☐ Unsure ☐

43. A high carbohydrate meal 2 – 4 hours pre exercise can lead to improvements in endurance performance. *(True or false?)*

True ☐ False ☐ Unsure ☐

44. Which of the following drinks contains the highest amount of carbohydrate? *(Please Tick one box)*

500 ml Coke ☐
500 ml Powerade ☐
500 ml full fat milk ☐
500 ml orange squash ☐
Unsure ☐

45. In general, which of the following meals would be recommended to eat 3 hours before training? *(Please Tick one box per question).*

a) Steak and chips	☐	or	Cous cous and tuna	☐
b) Mars bar and crisps	☐	or	Bread and jam sandwich	☐
c) Chicken sandwich	☐	or	Cornish Pasty	☐
d) Pasta and pesto	☐	or	Green leaf salad	☐

46. In general, what would be the best item to snack on in the 30 minutes pre exercise? *(Please tick one box per question).*

a) Jelly babies	☐	or	Peanuts	☐
b) Chocolate	☐	or	Banana	☐
c) Sunflower seeds	☐	or	½ a white bread jam sandwich	☐
d) Cereal Bar	☐	or	50g low fat crisps	☐

47. For a power athlete trying to increase muscle mass how much protein should they be eating per day (g/kg BM = grams per kilogram of body mass). *(Please tick one box).*

0.8 – 0.9 g.kg BM ☐
1.0 – 1.4 g.kg BM ☐
1.5 – 2.0 g.kg BM ☐
2.1 – 2.5 g.kg BM ☐
2.6 – 3.0 g.kg BM ☐
Unsure ☐

48. If using a whey protein supplement, how much protein (in grams) do guidelines state should be consumed in one serving? *(Please tick one box).*

10 – 17 g ☐
18 – 24 g ☐
25 – 30 g ☐
31 – 37 g ☐
38 – 45g ☐
Unsure ☐

49. If competing twice in one day, morning and evening:

a) When is the optimum time to eat after the first event? *(Please tick one box).*

0 – 45 minutes ☐
45 – 90 minutes ☐
90 – 125 minutes ☐
Eat after the second race. ☐
Unsure ☐

b) Is it more important to replace carbohydrate, protein or fat after the first event? *(Please tick one box).*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

50. When carbohydrate loading, what percentage of your diet should come from carbohydrate? *(Please tick one box).*

45% ☐
60% ☐
75% ☐
90% ☐
Unsure ☐

51. Ideally, what percentage body fat would a world-class athlete have in the following events? *(Please tick one box per discipline)*

Sprinter	Long Jumper	Marathon Runner
0 – 3 % ☐	0 – 3 % ☐	0 – 3 % ☐
4 – 8 % ☐	4 – 8 % ☐	4 – 8 % ☐
10 – 14 % ☐	10 – 14 % ☐	10 – 14 % ☐
16 – 20 % ☐	16 – 20 % ☐	16 – 20 % ☐
Unsure ☐	Unsure ☐	Unsure ☐

52. Is it always beneficial to have a isotonic sports drink in the 60 minutes pre event? *(Please tick one box)*

Yes ☐
No ☐
Unsure ☐

53. What food group is made up of amino acids? *(Please tick one box)*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

54. If an amino acid is termed 'essential' what does this mean? *(Please tick one box)*

You should consume this in your diet ☐
It is produced in the body ☐
Should eat it before you exercise ☐
Unsure ☐

55. Immediately post exercise is it best to consume high or low glycemic index carbohydrates? *(Please tick one box).*

High GI ☐
Low GI ☐
Unsure ☐

56. Salt tablets could be used as a preventative if you regularly suffer from cramp during exercise. *(Please tick one box)*

Agree ☐ Disagree ☐ Unsure ☐

57. Supplementing with caffeine will result in a decrease in performance in the following events? *(Tick as many as relevant).*

	True	False	Unsure
Sprinting	☐	☐	☐
Long Jump	☐	☐	☐
800 m	☐	☐	☐
Shot Putt	☐	☐	☐
Marathon	☐	☐	☐
All or the above	☐	☐	☐

58. Supplementing creatine has the same effect as supplementing with whey protein *(Please tick one box).*

True ☐ False ☐ Unsure ☐

59. In general, which athletic group would benefit from supplementing creatine monohydrate? *(Tick as many as relevant).*

Speed and power ☐
Endurance ☐
Unsure ☐

60. If using creatine how much is recommended you should take per day? *(Please tick one box).*

1 gram ☐
3 grams ☐
5 grams ☐
7 grams ☐
9 grams ☐
Unsure ☐

61. Which type of athlete, has research has shown, could benefit from taking sodium bicarbonate as a performance aid? *(Please tick one box)*

100 – 200 meters ☐
400 – 1500 meters ☐
5,000 – Marathon ☐
Throwers ☐
Unsure ☐

62. What are the physiological benefits of taking sodium bicarbonate? *(Please tick one box)*

Increase energy production ☐
Increase free radical removal ☐
Improve hydration status ☐
Maintain blood pH ☐
Increase maximal voluntary contraction ☐
Unsure ☐

Figure 4.2.2. Items selected and concluded as valid and reliable which comprise the final NKQA developed.

The full questionnaire can be found in Appendix Three.

4.3 RESULTS

The reliability and validity statistics for the final set of questions are displayed in Table 4.3.1 and Table 4.3.2. Response for the retest phase of the study was high with 51 of the original 53 (96%) participants from the NUT group, and 47 of the original 48 (98%) NONUT group completing the questionnaire for both test and retest phase. Three participants did not respond to the invitation to complete the questionnaire for a second time.

The NUT group achieved a significantly ($p < 0.0001$) higher score than the NONUT group in each of the subsections. In both groups the fat subsection was the highest scoring (84.9% and 58.2%, in NUT and NONUT, respectively $t = 11.728$, $p < 0.0001$), whilst the lowest mean scores were observed in the fluid subsection (72.8% and 40.0% in NUT and NONUT, respectively, $t = 9.723$, $p < 0.0001$). Overall the NUT group provided a correct answer for 80.4% of the questions, whereas the NONUT group answered less than half (49.6%) the questions correctly ($t = 20.90$, $p < 0.0001$).

Table 4.3.1. Nutrition knowledge sub-section and total score.

Sub-section and total score achieved (mean, SD and percentage (%)) on the nutrition knowledge questionnaire for athletes by the NUT and NONUT groups.

Nutrition knowledge sub-section (n)	NUT			NONUT		
	Mean	SD	Percentage (%)	Mean	SD	Percentage (%)
Carbohydrate (23)	19.1*	1.54	82.9	12.6	4.34	54.6
Protein (18)	13.9*	1.90	77.5	8.7	3.04	48.5
Fat (23)	19.5*	2.12	84.9	13.4	3.11	58.2
General NUT (31)	25.7*	3.60	82.9	16.3	3.53	52.6
Fluid (15)	10.9*	2.28	72.8	6.0	2.81	40.0
Sports nutrition (35)	27.9*	5.67	78.5	14.9	4.46	42.2
Total (145)	116.7*	10.8	80.4	71.9	10.63	49.6

** Scored significantly higher within category than the NONUT ($p < 0.001$). NUT = nutrition trained group, NONUT = no nutrition training group*

Table 4.3.2. Internal reliability of the NKQA.

Internal reliability of the NKQA for the first data collection, also test retest reliability and identical response rate over two data collection periods separated by three weeks.

Nutrition knowledge sub-section (n)	Internal reliability †	Test – retest correlation *	Identical responses from both tests (all) (%)	Identical responses from both tests (NUT) (%)	Identical responses from both tests NONUT) (%)
Carbohydrate (23)	.84	.95	91.0	94.5	87.5
Protein (18)	.81	.94	89.9	92.5	87.4
Fat (23)	.78	.97	93.5	93.4	93.6
General NUT (31)	.86	.92	87.7	90.8	84.5
Fluid (15)	.82	.91	84.0	84.6	83.3
Sport nutrition (35)	.92	.87	83.4	84.5	82.4
Total (145)	N/A	.98	88.0	89.8	86.1

*NUT = nutrition trained group, NONUT = no nutrition training group. † = Chronbach's alpha, * = Pearson's correlation significant at p < 0.05*

Strong test retest reliability (Table 4.3.2) was observed; the correlation for the total questionnaire was 0.98 ($p < 0.05$), all of the subsections produced a correlation ≥ 0.87 ($p < 0.05$). The internal reliability (Table 4.3.2) for each of the subsections achieved the psychometric requirements to determine reliability (Chronbach's $\alpha > 0.7$). The fat subsection produced the lowest alpha level at 0.78; the sport nutrition subsection produced the highest alpha level at 0.92.

The test retest results for all participants produced an identical response to the same question 88.0% of the time, with the nutrition group exhibiting an identical response of 89.8% of the time and the NONUT group 86.1% of the time. The carbohydrate subsection produced the highest number of identical responses in the nutrition group with 94.5%, whereas the fat subsection produced the highest number of identical responses with 93.7% for the non-nutrition group. The sports nutrition subsection produced the lowest number of identical responses, with 84.5% and 82.4% for the nutrition and non-nutrition groups respectively.

4.4 DISCUSSION

The aim of the present study was to develop a valid and reliable general and sport nutrition knowledge questionnaire that can be used as a practical tool to assess nutrition knowledge in track and field athletes.

The comprehensive and structured psychometric evaluation of the current questionnaire demonstrated strong reliability and validity. The NUT group (who had considerable training in the field of nutrition) produced > 30% ($p < 0.05$) more correct responses than the NONUT group (who had no prior exposure to nutrition training) throughout the questionnaire, such significant differences in the scores of the two groups is sufficient to assume construct validity (Cronbach, 1951). Equally, the test retest correlation (0.98, $p < 0.05$) demonstrated satisfactory internal reliability (Kline, 2007a) and internal consistency can also be assumed as each subsection produced a Chronbach's alpha value > 0.7 (Kline, 2007a). Therefore the H_0 was rejected; a new tool for the assessment of general and sport nutrition knowledge in track and field athletes was established.

The length of the questionnaire was adequate to attain the relevant information needed to draw conclusions about the responders' nutrition knowledge, but not too long to reduce compliance. It has been suggested that questionnaire length may impact participant response, with longer questionnaires reducing compliance (Galesic and Bosnjak, 2009), however in a recent meta-analysis (Rolstad et al., 2011) only three of twenty-five studies investigating questionnaire length demonstrated a weak correlation between questionnaire length and participant response. With 14 pages of questions and an average completion time of 15 minutes 20 seconds the questionnaire is of a moderate duration, the test re-test completion rate of the questionnaire was high, with 98 of the original 101 participants completing the questionnaire on both occasions. Thus, despite the questionnaire being longer than other nutrition knowledge questionnaires it is reasonable to suggest that the length will not impact completion and accuracy.

The test – retest correlation was high and consistent across all subsections ($r = 0.93 \pm 0.04$, range 0.87 – 0.97), and the total test – retest correlation for all 145 questions

was 0.98. With such a strong test – retest reliability the questionnaire provides a tool, which if administered over time (> 3 weeks between administrations) could be used to assess the effectiveness of an intervention or nutrition education programme.

An inability to answer questions correctly can increase respondent bias (Meisenberg and Williams, 2008), reducing the accuracy of a questionnaire. To control for this the questionnaire included a briefing paragraph detailing the importance of not guessing, also the presence of an 'unsure' answer choice provided the responder with an answerable option. The results from both groups within this study produced a higher number of identical responses across the test – retest period (89.8% vs. 86.1%; NUT vs. NONUT), thus it is fair to assume that the respondent bias of the questionnaire was low, indicative of a low percentage of questions with a guessed answer. A slightly lower test re-test identical question response was observed in the NONUT group relative to the NUT group, which could be attributed to lesser knowledge, confidence and more indecisiveness in the answers chosen (Meisenberg and Williams, 2008). However the NONUT group still produced a very high identical response rate of 86%, as such this is of little concern to the validity and reliability of the questionnaire.

Previously, the most comprehensive psychometrically validated sport nutrition knowledge questionnaire developed (Zinn et al., 2005) lacked a broad general nutrition subsection, raising concerns over conclusions drawn about the participants' general nutrition knowledge. The current questionnaire includes 32 questions (95 parts) addressing macronutrients and general nutrition, as such the range and quantity of these questions are similar to that of a previously validated general nutrition knowledge questionnaire for adults (Parmenter and Wardle, 1999), additionally a further 33 questions (50 parts) address hydration and sport specific nutrition, thus providing sufficient information for conclusions to be drawn about the specific areas of the individuals general and or sport nutrition knowledge. The range of subsections represents a greater depth of assessment than any current available tools.

A systematic review from Heaney et al. (2011) found a weak positive association ($r < 0.44$) between nutrition knowledge and improved dietary intake in athletic populations, and the evidence supporting was inconclusive, as such the relationship between enhanced nutrition knowledge and improved dietary choices is unclear. Furthermore,

the validity of the sport specific nutrition knowledge assessment was inconsistent within these studies. The questionnaire should be used as a tool to investigate the knowledge of the athlete, not as a complete solution to comprehend nutrition knowledge and dietary intake of the responder.

To date, this questionnaire is the most robust measure of nutrition knowledge in track and field athletes; however it is important to note that this questionnaire was developed to assess the knowledge of UK Track and Field athletes. If used for a different athletic population the questionnaire is reduced in validity, as such if this NQKA is selected to be used in a different athletic population it would be recommended to make subtle, relevant changes to the questions and test the validity prior to use.

4.5 CONCLUSION

The NKQA provides a psychometrically validated and reliable tool for the assessment of general and sport nutrition knowledge in UK track and field athletes. The included questions cover a broad range of general and sport nutrition topics consequently the differentiation in nutrition knowledge subsections are distinguishable from the results. The questionnaire developed in this Chapter will be used to assess nutrition knowledge in subsequent studies contributing towards this thesis.

5 NUTRITION KNOWLEDGE, DIETARY INTAKE AND METABOLIC TYPE OF UK TRACK ATHLETES.

Abstract

Background: Optomising nutritional intake can enhance health and sporting performance, a number of factors influence dietary intake, nutrition knowledge being one of these. Furthermore, personalised nutrition strategies are being implemented to provide bespoke nutrition solutions for the individual, one such commonly used programme, the Metabolic Typing Questionnaire is yet to be explored in a research setting. Thus the aims of the chapter were to examine the current dietary habits and assess the nutrition knowledge of UK track and field and also investigate the Metabolic Typing Questionnaire as a tool to prescribe bespoke nutritional strategies. ***Method:*** Eighty-eight participants were recruited and classified as either endurance athletes (END, n = 29), power athletes (POW, n = 30) or control (CON, n = 29) and the participants completed a metabolic typing questionnaire, food diary and NKQA at two distinct time-points (October/November, and March/April). ***Results:*** The main findings from this Chapter demonstrate that 94.3% of the participants were highlighted as metabolic type B, athletes have greater nutrition knowledge than non-athletes (67.1 ± 3.2 vs. 65.0 ± 2.3 vs. 45.4 ± 1.8 %, END, POW, CON. $p < 0.001$). No difference in dietary macronutrient ration is apparent between END and POW athletes (carbohydrate = 413.4 ± 98.4 vs. 399.2 ± 113.7 g, protein = 111.8 ± 26.4 vs. 110.8 ± 37.5, fat = 75.0 ± 15.2 vs. 78.4 ± 30.7 g). No relationship exists between nutrition knowledge and dietary intake in UK track and field athletes ($r^2 = 0.003$, $p = 0.63$). **Conclusion:** The results of a 65-questions metabolic typing questionnaire is not sensitive enough to prescribe individualised diets for healthy or an athletic population. UK track and field athletes possess greater nutrition knowledge than a matched control group, however no difference exists between nutrition knowledge and the quality of dietary intake.

5.1 Introduction

As described in Chapter 2.1 it is well established that optimising nutritional strategies can help improve health and sporting performance (Burke and Deakin, 2010), whilst inadequate intake can be detrimental (Gleeson and Bishop, 2000). Consequently an abundance of nutritional research exists. In an to attempt to formulise a set of nutrition guidelines for athletes the ACSM publish regular position statements (Thomas et al., 2016). However in general, nutritional recommendations are event specific; for example speed and power athletes should consume a high protein diet and endurance athletes a higher carbohydrate diet (Tipton et al., 2007). More recent advances in science have opened up the possibility for nutrition to be personalised to an individual's characteristics; not just the disease state, goal or athletic event (Joost et al., 2007). Once such tool for individual dietary prescription is the metabolic typing questionnaire. The method utilises a 65-item self-assessment questionnaire to prescribe a suitable diet to match metabolic type, designed to restore metabolic homeostatic balance and optimise health (as previously described in 2.1.3). To date, no research exists investigating the efficacy of the questionnaire. Power and endurance athletes are an ideal population to investigate the sensitivity of the questionnaire due to the phenotypic difference and physiological characteristic of the athletes, it is hypothesised that difference in phenotype may be associated to metabolic type.

In order to make healthy food choices it is important to have an internalised and comprehensive structure of nutritional knowledge (Rasanen et al., 2003). A person may master some aspects of nutrition, e.g. from the point of view of basic nutritional sciences or nutrition-related diseases, however if understanding the connections between different nutritional facts is incomplete the translation of this knowledge into correct food decisions may not be established (Cotugna et al., 1992). Despite significant research into optimal dietary intake, athletes' diets are commonly reported as being nutritionally inadequate (Heaney et al., 2011), the underlying reasons for this are unclear.

Nutrition knowledge refers to knowledge of concepts and processes related to nutrition and health; this may be extended to knowledge about specific nutrition topic which

impacts an identifiable outcome such as sport nutrition for athletic performance (Miller and Cassady, 2015). In order to make healthy food choices it has been stated that an understanding of nutrition knowledge must be internalised (Rasanen et al., 2003). Numerous nutrition knowledge questionnaires exist and selecting the correct tool is essential for reliable assessment (Refer to Chapter 4 for a detailed description of the processes and the steps taken to create a valid and reliable questionnaire for this experimental Chapter). However, as described in detail in Chapter 4, the relationship between nutrition knowledge and nutrition intake is not robust in athletes, with Heaney et al. (2011) stating a weak positive correlation (r = 0.44). This relationship is yet to be explored in UK track and field athletes.

5.1.1 Aims and Objectives

There are two primary aims of this piece or research, the first to investigate the effectiveness of the MT questionnaire as a tool for dietary prescription, the second to investigate the relationship between nutritional intake and nutrition knowledge within UK track athletes.

5.1.2 Experimental hypotheses.

i) (**H_1**) A commercially available metabolic typing questionnaire will accurately differentiate metabolic type between power and endurance athletes.
(**H_0**) A commercially available metabolic typing questionnaire will not be able to differentiate metabolic type in power and endurance athaltes.
ii) (**H_2**) Athletes will have greater nutrition knowledge than the non-athletic population.
(**H_0**) No difference in nutrition knowledge will be observed between athlete and non-athlete populations.
iii) (**H_3**) A positive relationship exists between dietary intake and nutrition knowledge.
(**H_0**) No relationship exists between dietary intake and nutrition knowledge.
iv) (**H_4**) A distinct difference in dietary carbohydrate and protein macronutrient intake will be observed between endurance and power athletes.
(**H_0**) No difference in dietary carbohydrate or protein macronutrient intake will be observed between endurance and power athletes.

5.2 Methods

5.2.1 Participants

Ninety-two (42 female, 50 male) participants volunteered for this study. Sixty-one UK track athletes were recruited at athletic events and via magazine advert (Appendix Five), 29 students (undergraduate and postgraduate) with no athletic background or nutrition training were recruited through university adverts acted as the control (CON) group. The inclusion criteria for the athletic population included being ranked within the top 150 senior athletes in the UK for their event and currently undertaking ≥ 4 event specific training sessions per week. The average UK national ranking of the participants at the end of the 2012 season was 40.3 ± 29.6 (mean ± SD, range 2nd – 133rd). Each athlete provided personal bests for self selected best two events, which were verified, along with national ranking on the www.thepowerof10.info website, and used for grouping allocation (Table 5.2.1).

Table 5.2.1. Athlete grouping criteria.

Group	Primary event	Second event
Power (POW)	≤ 800 m	≤ 400m
Endurance (END)	≥ 800m	≥ 1,500m

Ninety-two participants completed the first phase of data collection (END n = 31, POW n = 32, CON n = 29), with 88 (48 male, 40 female) completing both phases of data collection (END n = 29, POW n = 30, CON n = 29, Table 5.2.2). All four participants who dropped out were due to non-response from second questionnaire and were removed from all data analysis. Informed consent was attained prior to the completion of the first questionnaire. The study was approved by the University of Hertfordshire Life and Medical Sciences ethics committee, protocol approval number: LS/4/5/11P.

Table 5.2.2. Self-reported participant characteristics.

	Age (years)	Weight (kg)	Height (cm)	BMI
END (n = 29)	24.5 ± 4.8	62.6 ± 9.3	173 ± 1.1	20.9 ± 2.9
POW (n = 30)	25.4 ± 5.1	69.2 ± 10.4	174 ± 0.8	22.9 ± 2.4
CON (n = 29)	24.8 ± 4.4	72.5 ± 13.9	169 ± 1.3	25.2 ± 3.6

5.2.2 Study Protocol

Two questionnaires, a nutrition knowledge questionnaire for athletes (NKQA) (As described in Chapter 4) and a metabolic typing questionnaire (MTQ) (Walcott and Fahey, 2000), along with a 3-day food diary were distributed to the participants at two time points throughout the athletic season: early season (October and November 2011) and the start of the competitive season (March and April 2012). Participants completed both questionnaires either online, or by hand and returned to the lead researcher. The food diaries were either emailed or downloaded by participants and returned via email or post.

5.2.3 Experimental protocol.

The two questionnaires (NKQA and MTQ) were uploaded onto a secure clinical research online survey platform (Bristol Online Surveys, University of Bristol). The link to the questionnaires and instructions for completion were emailed to each participant. The link directed the participant to the NKQA. Once completed, the questionnaire had to be completed in one sitting, the participant was provided with an opportunity to either pause or complete the MTQ immediately. If the participants had not completed the MTQ within 48 hours of the NKQA a reminder email was sent. Upon completion of the MTQ a link to download the food diary was provided. The participants were required to complete a 3-day food diary using household measures on two consecutive weekdays and one weekend day. The participants were contacted ~ 5 months later via email and requested to complete the process again.

5.2.4 Nutrition Knowledge Questionnaire

Preceding the nutrition knowledge questionnaire were three belief questions referring to perceived habitual dietary intake. The nutrition knowledge questionnaire used in this experimental Chapter was the NKQA developed in Chapter 4 of this thesis. The NKQ was scored according the number of correct answers. If the participant answered correctly the question was scored as 1, if the participant answered incorrectly, ticked unsure or did not answer the question, the question was scored as 0.

5.2.5 Metabolic Typing Questionnaire.

Metabolic type was assessed using a commercially available 65-question metabolic typing questionnaire (Walcott and Fahey, 2000) which was adapted for a UK population (Appendix One). The adaptations were made to replace common American foods with similar, more familiar, British food items for a UK population.

The metabolic typing questionnaire results were calculated according to the method described by Walcott and Fahey (2000). Each answer choice was classified as either and A, B or C. The subtotal of each answer category is then calculated and metabolic type concluded:

- If the number of A answers was 5 or more higher than both B and C, then the participant was classified as a '*Carbohydrate Type*' (Type A).
- If the number of C answers was 5 or more higher than both A and B, then the participant was classified as a '*Protein Type*' (Type C).
- If the number of B answers was 5 or more higher than both A and C, then the participant was classified as a '*Mixed Type*' (Type B).
- If neither A, B or C were 5 or more higher than both of the other two then the participant was classified as a '*Mixed Type*' (Type B).

5.2.6 Diet Quality Index (DQI)

Diets were assessed for quality using a diet quality index adapted from Haines et al. (1999) (Table 5.2.3). Dependent on a pre-defined scoring criteria, total fat, saturated fat, dietary cholesterol, calcium, magnesium, vitamin D, vitamin B12, vitamin C, iron and sodium intakes were scored either 0, 5 or 10 to provide a total DQI score (0 – 100).

Table 5.2.3. DQI components and scoring criteria.(Adapted from Haines et al. (1999)).

Component	Score	Scoring Criteria
Total fat	0 – 10 Points	≤ 30% = 10 > 30, ≤ 40% = 5 > 40% = 0
Saturated fat	0 – 10 Points	≤ 10% = 10 > 10, ≤ 13% = 5 > 13% = 0
Dietary cholesterol	0 – 10 Points	≤ 300 mg = 10 > 300, ≤ 400 mg = 5 > 400 mg = 0
Calcium intake	0 – 10 Points	≥ 800 mg = 10 ≥ 600, < 800 mg = 5 < 600 mg = 0
Magnesium intake	0 – 10 Points	≥ 350 mg = 10 ≥ 263, < 350 mg = 5 < 263 mg = 0
Vitamin D intake	0 – 10 Points	≥ 400 IU = 10 ≥ 300, < 400 IU = 5 < 300 IU = 0
Vitamin B12 intake	0 – 10 Points	≥ 1.3 mg = 10 ≥ 0.97, < 1.3 mg = 5 < 0.97 = 0
Vitamin C intake	0 – 10 Points	≥ 60 mg = 10 ≥ 45, < 60 mg = 5 < 45 mg = 0
Iron intake	0 – 10 Points	≥ 18 mg = 10 ≥ 13.5, < 18 mg = 5 <13.5 mg = 0
Sodium intake	0 – 10 Points	≥6000 mg = 0 ≥4500, <6000 mg = 5 <4500 mg = 10

5.2.7 Dietary Intake

Dietary intake was recorded and analysed as previously described in Chapter 3.1. The following outputs were exported for analysis: daily calorie intake (kcal), water (ml), carbohydrate (g), protein (g), fat (g), alcohol (g), saturated fat (g), cholesterol (mg), calcium (mg), magnesium (mg), iron (mg), sodium (mg), vitamin D (IU), vitamin B12 (mg) and vitamin C (mg).

5.2.8 Statistics

Data were analysed using SPSS version 20.0.0 for Macintosh (IBM, Armonk, NY). Kolmogorov-Smirnov tests of normality to assess the null hypothesis that data for within-subjects analysis was normally distributed. However it should be noted that the ANOVA procedure is robust to slight violations of normality within a dataset (Field, 2013). Mauchly's sphericity test was used to assess the assumption of sphericity within repeated-measures effects. Unless stated otherwise Mauchly's test was insignificant ($p > 0.05$), therefore the assumption of sphericity was accepted

A Pearson's correlation was used to assess for a time-point correlation in both the MTQ and NKQ, and assess the relationship between NKQA score and DQI. A one-way analysis of variance (ANOVA) was used to assess between group differences in the NKQ (total and sub-section) score, MTQ score and macronutrient intake, if significance was identified a Bonferroni post hoc test was used to identify where the difference lies. A paired sample t-test was used to identify difference between actual and predicted dietary intake. All results are presented as mean ± SD (unless stated) and a significance level of $p \leq 0.05$ was accepted throughout.

5.3 Results

As detailed below in Figure 5.3.1, Figure 5.3.2 and Figure 5.3.3 (A, B, C, and D) along with Table 5.3.1 a strong correlation exists and/or no time point difference was observed between the two collection points for all variables measured. As such, for

ease of discussion and the flow of analysis, the results section will provide description and commentary on the data from the first phase of collection.

5.3.1 Time point analysis of the variables.

A strong correlation of $r^2 = 0.78$ ($p = 0.0001$) was observed in the total score achieved in all participants from phase one (81.9 ± 16.6) and phase two (84.7 ± 17.7) of data collection, separated by ~ 5 months (Figure 5.3.1).

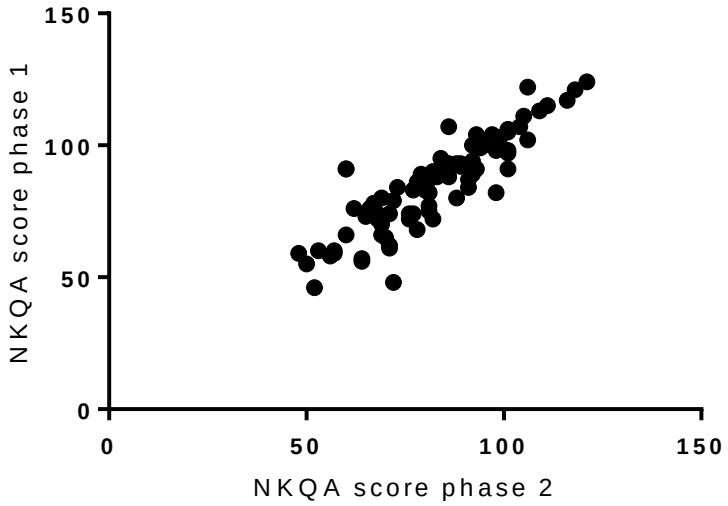


Figure 5.3.1. The NKQA score correlation between phases one and phase two of data collection.

($r^2 = 0.78$, $p = 0.0001$).

A significant ($p < 0.0001$) positive correlation was observed in each answer category from the metabolic typing questionnaire between the two phases of data collection (Table 5.3.1), also no difference was observed between the total number of answers each category received at phase one and phase two (A; 11.8 ± 4.6 vs. 12.3 ± 5.1, B; 29.5 ± 6.3 vs. 29.5 ± 6.4, C; 14.5 ± 5.0 vs. 13.1 ± 4.7, Un; 9.2 ± 2.9 vs. 10.1 ± 4.6).

Table 5.3.1. The correlation of MTQ answers between phase one and two.

Answer (category)	Phase 1 – Phase 2 correlation	
	Correlation (r^2)	Significance (*p* value)
A	0.547	< 0.0001
B	0.562	< 0.0001
C	0.590	< 0.0001
Un	0.422	< 0.0001

A significant ($p < 0.0001$) time point correlation was observed in grouped DQI score attained between phase one (73.8 ± 11.7) and phase two (72.7 ± 12.2) (Figure 5.3.2, $r^2 = .63$). Additionally no within group time point difference in DQI score was observed (END; 77.8 ± 9.0 vs. 76.1 ± 11.5, POW; 76.9 ± 12.1 vs. 76.4 ± 11.7, CON; 67.6 ± 13.9 vs. 65.7 ± 13.5, $p > 0.05$) (Figure 5.3.2).

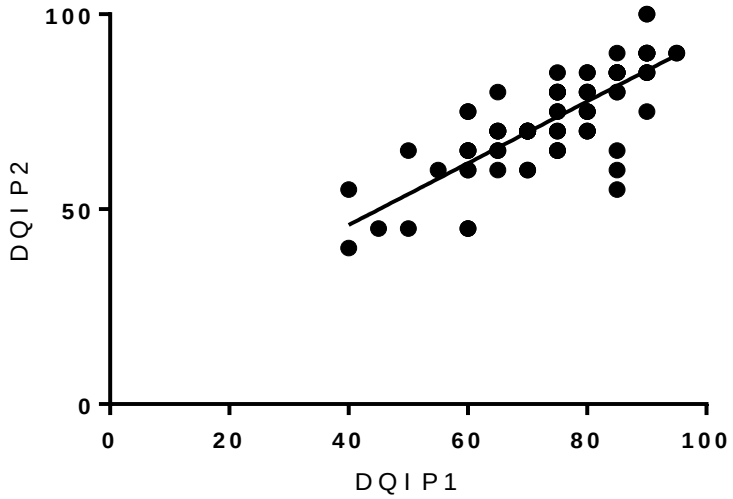


Figure 5.3.2. Correlation in the DQI score achieved in phase one and phase two of data collection.

($r^2 = .63$, $p < 0.0001$)

A significant ($p < 0.0001$) time point correlation was observed in macronutrient (%) and calorie (kcal) intake between phase one and phase two within all groups (PRO; 16.7 ± 4.8 vs. 16.4 ± 3.9, FAT; 27.0 ± 6.0 vs. 26.6 ± 5.4, CHO; 55.8 ± 7 vs. 56.3 ± 6, kcal; 2422 ± 646 vs. 2389 ± 566) (Figure 5.3.3 A – D).

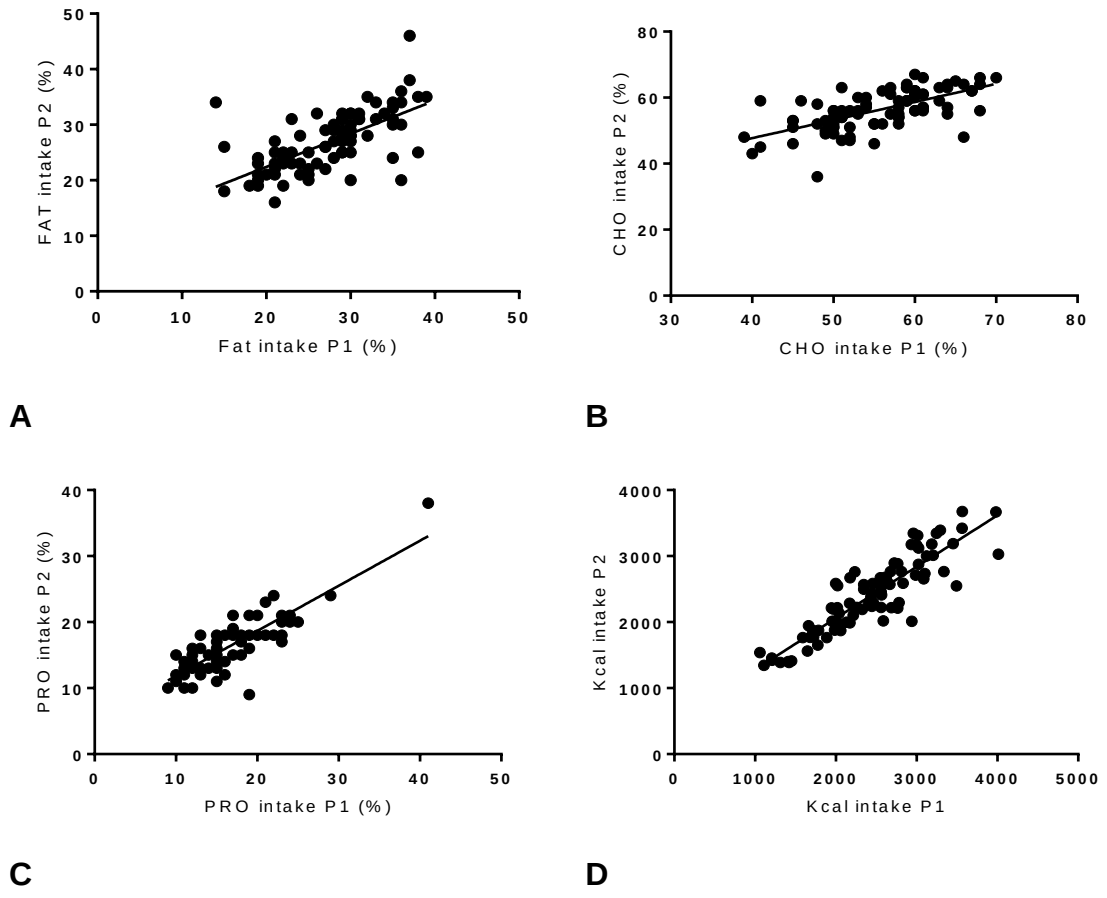


Figure 5.3.3. The correlation in macronutrient intake (%) and total calorie intake between phase one (P1) and phase two (P2) of data collection.

*Macronutrient intake calculated using a 3-day food diary using household measures. FAT intake; $r^2 = 0.44$, $p < 0.0001$ (**A**), CHO intake**,** $r^2 = 0.41$, $p < 0.0001$ (**B**), PRO intake**,** $r^2 = 0.69$, $p < 0.0001$ (**C**) and Kcal intake**,** $r^2 = 0.79$, $p < 0.0001$ (**D**).*

5.3.2 Metabolic typing questionnaire

All three groups produced a similar response in respect to unanswered (Un) questions (9.4 ± 0.4, 9.2 ± 0.4, 9.1 ± 0.7) and questions answered 'B' (29.2 ± 1.2, 29.5 ± 1.0, 29.8 ± 1.3) within the MTQ. Additionally no significant differences were observed between the two athletic groups in any answer category. A main effect (f = 3.728, p = 0.028) was apparent in the 'A' and 'C' (f = 4.099, p = 0.02) answer categories, with the CON group answering a significantly greater number of questions 'A' and 'C' relative to the POW group (10.6 ± 0.7 vs. 13.6 ± 1.0, p = 0.032 and 15.7 ± 0.9 vs. 12.5 ± 0.7, p = 0.25), no other differences were observed.

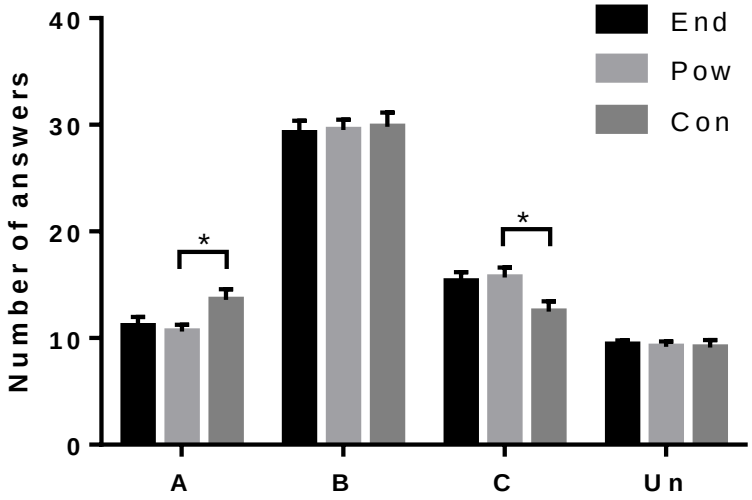


Figure 5.3.4. The mean answer choice from the MTQ for each group *(Mean ± SEM).*

** Denotes significance between POW and CON groups (p < 0.05).*

Using the grouping criteria as outlined in Section 5.2.5, 94.3% (n =83 of 88) of the participants were identified as type B (mixed type), only 2 participants (both from the control group) were identified as type A (carbohydrate type) and 3 participants as type C (protein type).

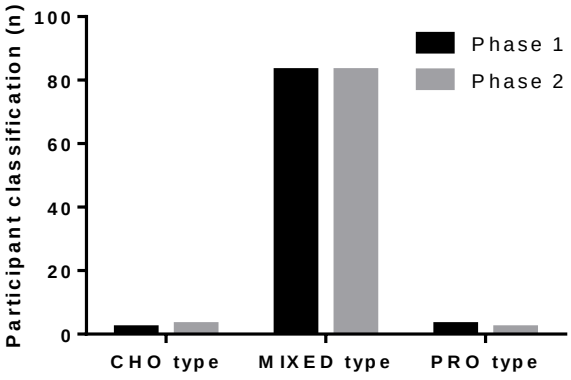


Figure 5.3.5. Metabolic type classification using the Wolcott and Fahey (2000) method.

5.3.3 Metabolic type and dietary intake

The results of the phase one data collection identified 2 participants (2.3%) as 'type A', 3 (3.4%) as 'type C' and 83 (94.3%) as 'type B'. Due to the low numbers identified as type A and C it was not possible to complete statistical analysis, the following descriptive results are reported. The habitual dietary intake of the participants who highlighted as 'type A' (carbohydrate type) consisted of 57% carbohydrate, 19% protein and 24% fat, compared to the 56% carbohydrate, 17% protein and 27% fat for 'type B' (mixed typed) and 55% carbohydrate, 14% protein and 31% fat for 'type C' (protein type) respectively.

5.3.4 Nutrition Knowledge Questionnaire.

A main effect was observed ($f = 13.238$, $p < 0.0001$) in total nutrition knowledge score, determined by the completion of the NKQA developed in Chapter 4. Both the END ($p < 0.001$) and POW ($p < 0.001$) groups scored significantly higher compared to the CON group; no difference was observed between athletic groups (61.1 ± 2.3 vs. 59.7 ± 1.8 vs. 48.6 ± 1.5%) (Figure 5.3.6).

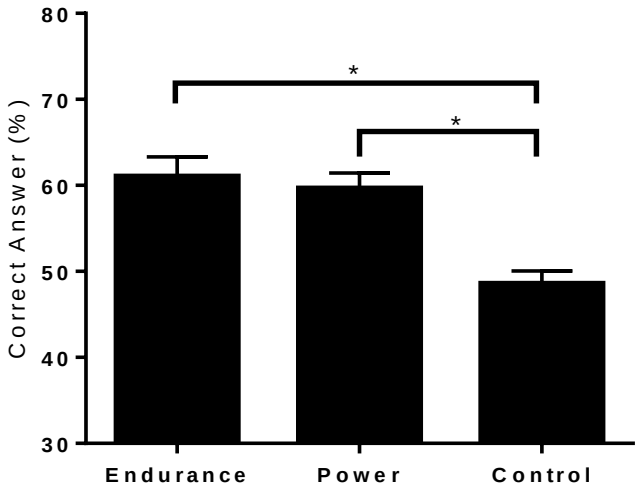


Figure 5.3.6. Overall NKQ score for each group.

** Denotes significance difference to control (p < 0.0001)*

A main effect was observed with both athletic groups scoring higher than the control group in the following sub sections on the NKQA: protein (END; 67.1 ± 3.2 vs. POW; 65.0 ± 2.3 vs. CON; 45.4 ± 1.8%, $f = 22.534$, $p < 0.001$), fat (END 67.5 ± 3.4 vs. POW 65.8 ± 2.9 vs. CON 54.6 ± 2.0, $f = 6.094$, $p = 0.003$), general nutrition (END 62.7 ± 2.2 vs. POW 61.7 ± 2.1 vs. CON 50.2 ± 1.9, $f = 11.007$, $p < 0.001$) and sport nutrition (END 56.7 ± 2.6 vs. POW 53.8 ± 1.8 vs. CON 42.0 ± 2.7%, $f = 10.447$, $p < 0.001$) (Figure 5.3.7). No significant difference was observed in the carbohydrate (END; 59.4 ± 4.2 vs. POW; 59.0 ± 3.6 vs. CON; 55.7 ± 3.3%, $f = 0.758$, $p = 0.278$) or hydration (END; 53.3 ± 3.6 vs. POW; 54.2 ± 3.3 vs. CON; 44.6 ± 3.6, $f = 2.319$, $p = 0.105$) knowledge subsections. Interestingly, both athletic groups scored lowest in the sections specific to sport, hydration and sports nutrition.

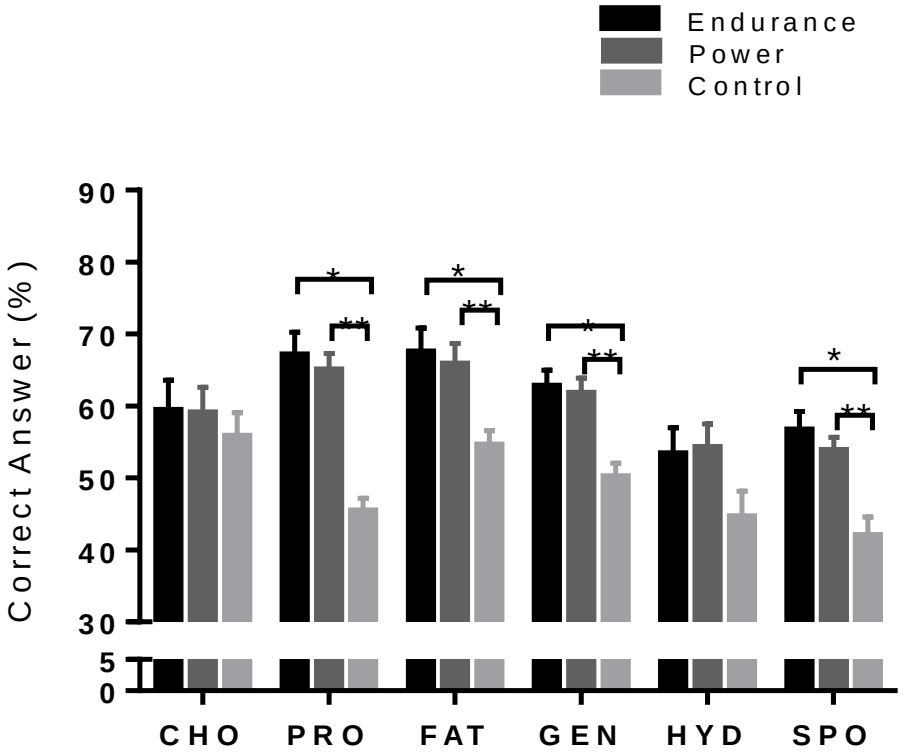


Figure 5.3.7. NKQ sub-category score from the END, POW and CON groups.

** Denotes significance between the END and CON groups (p < 0.05), ** denotes significance between the POW and CON groups (p < 0.05) (mean ± SD).*

No significant between group differences were observed in dietary carbohydrate (END; 57.2 ± 5.82, POW; 56.6 ± 7.8, CON; 53.5 ± 7.0%, $f = 2.499$, $p = 0.088$), protein (END; 16.9 ± 4.2, POW; 17.2 ± 6.0, CON; 16.2 ± 3.9%, $f = 0.357$, $p = 0.701$) or alcohol (END; 0.4 ± 1.0, POW; 0.3 ± 0.7, CON; 0.6 ± 1.1%) consumption as a percentage of macronutrient intake. The control group consumed a significantly greater amount of dietary fat compared to both athletic groups ($p < 0.05$, END; 25.4 ± 4.5, POW; 26.3 ± 6.8, CON; 29.2 ± 6.0%, $f = 3.556$, $p = 0.033$) (Figure 5.3.8, A, B, C and D). With regards to absolute dietary intake a significant group effect was observed in carbohydrate ($f = 17.235$, $p = 0.000$), protein ($f = 112.361$ $p = 0.000$), fat ($f = 3.439$, $p = 0.037$) and total calories ($f = 17.170$, $p = 0.000$), with the control group consuming significantly less carbohydrate ($p = 0.000$), protein ($p = 0.000$), and calories ($p < 0.0001$) compared to both athletic groups. The control group also consumed significantly less fat ($p = 0.014$) than the power group (Table 5.3.3). No difference was observed between the two athletic groups.

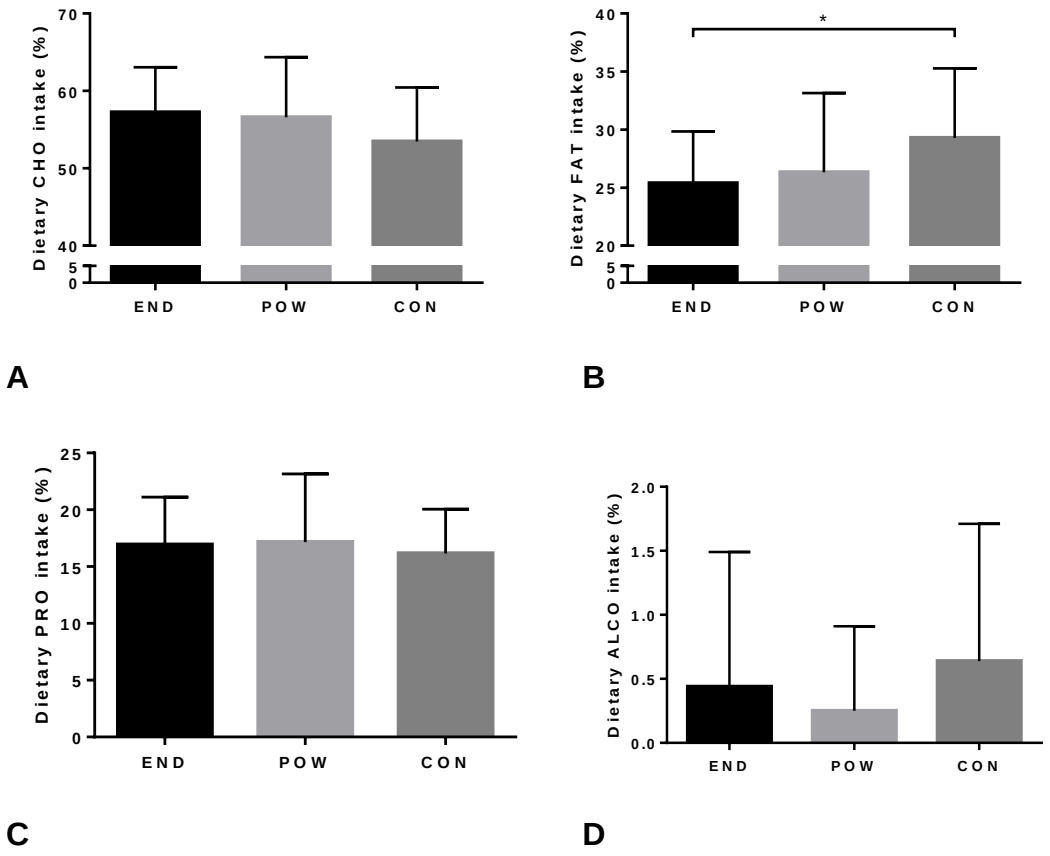


Figure 5.3.8. Mean macronutrient intake (%).

*Mean macronutrient intake (%), collected by a 3-day food diary using household measure during October – November 2011. END = endurance athletes, POW = power athletes, CON = control. Mean CHO intake (**A**), Mean FAT intake (**B**), Mean PRO intake (**C**) and Mean ALCO intake (**D**) (mean ± SD).* Denotes significance between END and CON (p < 0.05).*

Table 5.3.2 Habitual dietary intake represented as mean macronutrient grams (g), relative to body weight (g•kg^{-1}) and total calorie intake (kcal).

		END	POW	CON
CHO	(g)	413.4 ± 98.4 ¥	399.2 ± 113.7 ¥	275.4 ± 53.5
	g•kg^{-1}	6.6	5.8	3.8
FAT	(g)	75.0 ± 15.2	78.4 ± 30.7 *	63.1 ± 29.4
	g•kg^{-1}	1.2	1.1	0.9
PRO	(g)	111.8 ± 26.4 ¥	110.8 ± 37.5 ¥	76.9 ± 26.0
	g•kg^{-1}	1.8	1.6	1.1
Calories	(kcal)	2685 ± 447 ¥	2642 ± 663 ¥	1931 ± 516

** = significantly different from control group (p < 0.05). ¥ = significantly different from control group (p < 0.001)*

5.3.5 Predicted vs. actual dietary intake

All groups accurately predicted carbohydrate intake, with no significant difference between predicted intake (as a percentage of total intake) and actual dietary intake (as calculated by diet records) (Figure 5.3.9 A – C). The END and POW groups significantly over predicted protein intake by 9.8 % and 13.3 % (END; 26.7 ± 1.4 vs.16.9 % ± 0.8%, t = 6.084, p = 0.000, POW; 29.5 ± 1.7 vs. 17.2 ± 1.1%, t = -2.526, p = 0.014) and under predicted fat intake by 5.5 % and 4.4 % (END; 5.5 %; 19.8 ± 2.0 vs. 25.4 ± 0.8%, t = -2.608, p = 0.012, POW; 4.4 %; 21.9 ± 1.5 vs. 26.3 ± 1.4%, t = 6.196, p = 0.000) respectively (Figure 5.3.9). Whereas in the control group no significant difference was observed between predicted and actual fat intake (26.7 ± 1.1 vs. 29.3 ± 1.1), but a 9.9% over estimation of dietary protein intake was observed (26.0 ± 1.4 vs. 16.2 ± 0.7%, t = 6.471, p = 0.000, Figure 5.3.9 C).

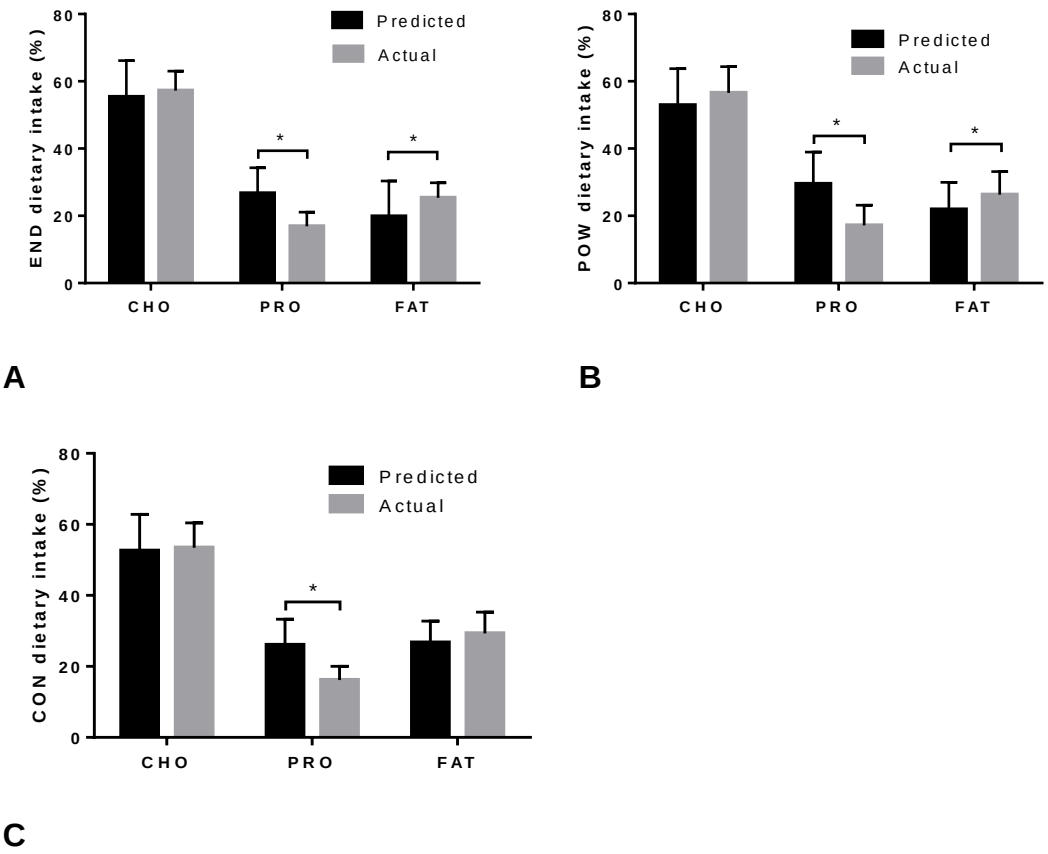


Figure 5.3.9. Mean predicted vs. Actual dietary intake

*Mean macronutrient intake as calculated by a 3-day food diary using household measures. END athletes (**A**), POW athletes (**B**),CON participants (**C**). * denotes significant difference between actual and predicted (p < 0.05) (mean ± SD)*

5.3.6 Diet quality.

A group main effect was observed in diet quality (f = 5.675, p = 0.005). The quality of diet for both athletic groups was significantly (END; 10.1% p = 0.006, POW; 8.4%, p = 0.03) greater than the control group with regards to DQI score (END = 77.7 ± 9.0, POW = 76.0 ± 12.1, CON = 67.6 ± 2.6) (Figure 5.3.10). No relationship was observed between DQI and NKQA score (Figure 5.3.11 A-D).

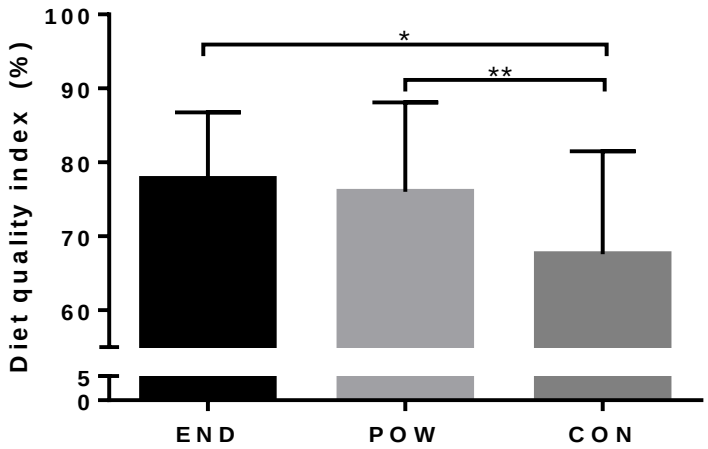


Figure 5.3.10**.** Diet quality index score (DQI) for each group.

*Calculated from a 3-day food diary using household measures. * denotes significance between END and CON p = 0.006. ** Denotes significance between POW and CON p = 0.030.*

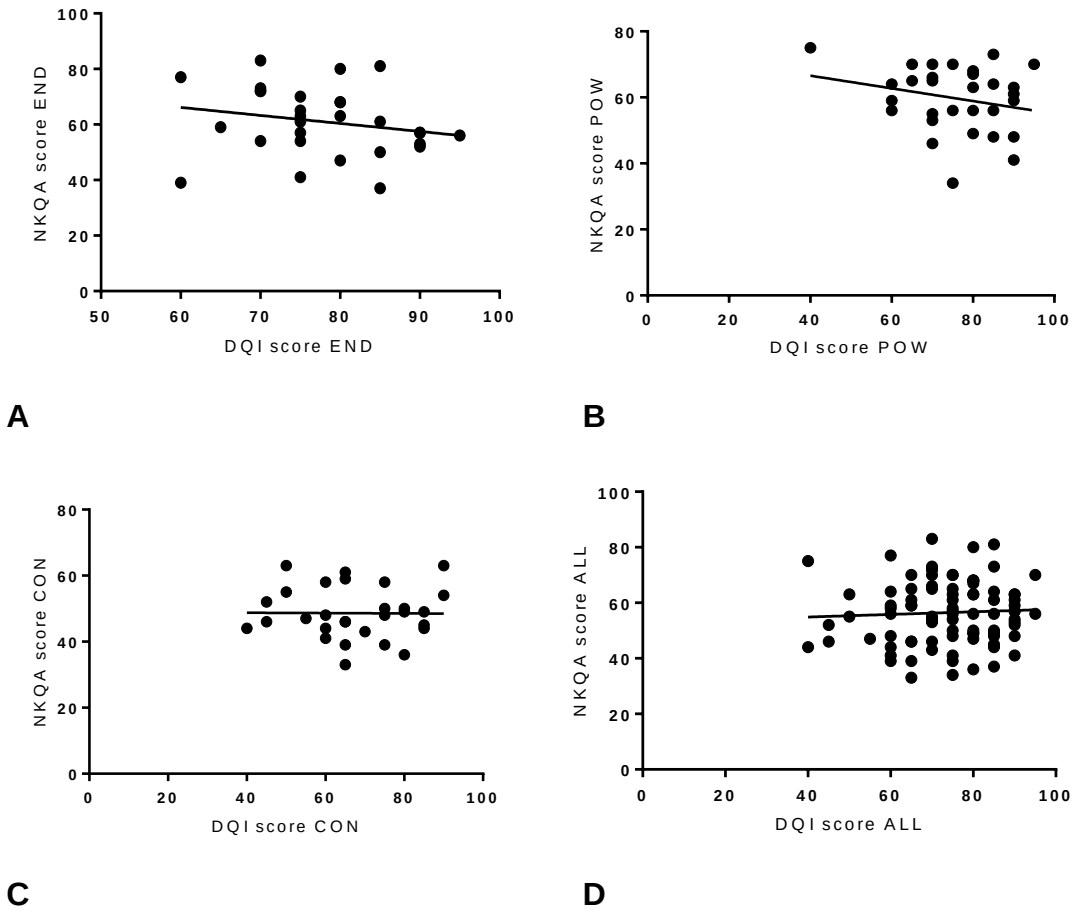


Figure 5.3.11. Relationship between nutrition knowledge and diet quality,

*Relationship between nutrition knowledge, measured using the nutrition knowledge questionnaire for athletes (NKQA), and diet quality, measured using the diet quality index (DQI) for each group (END = endurance athletes, POW = power athletes, CON = control group) and all participants grouped together (ALL). END; $r^2 = 0.05$, $p = 0.27$ (**A**), POW; $r^2 = 0.06$, $p = 0.21$ (**B**), CON**:** $r^2 = 0.00008$, $p = 0.96$ (**C**) and ALL; $r^2 = 0.003$, $p = 0.63$ (**D**).*

5.4 Discussion

With regards to the research hypotheses, the following can be summarised:

- It was hypothesised that a commercially available metabolic typing questionnaire is able to identify metabolic type and prescribe a diet personalised to meet the metabolic needs of the individual. As measured by the outcome of a 65-item questionnaire, 94.3% of the participants were highlighted as type B (mixed type). Therefore $\mathbf{H_0}$ is accepted, the questionnaire is not able to prescribe individualised diets specific to metabolic function. Additionally no association was observed between dietary intake of the different athletic groups and metabolic type. Therefore it can be assumed that, unless all athletes consume the same diet, the MTQ is not sensitive enough for individualised dietary intake in athletes.
- It was hypothesised that the athletic population have greater nutrition knowledge than the non-athletic control group. In rejection of $\mathbf{H_0}$, as measured by the NKQA, both athletic groups scored significantly higher than the control group on the overall NKQA, athletes do have greater nutrition knowledge than non-athletes.
- It was hypothesised that a positive relationship exists between dietary intake and nutrition knowledge. Although both athletic groups scored significantly higher than the control group for DQI and NKQA, an r^2 value of 0.003 was demonstrated when correlating DQI and NKQA score across all participants. Thus, although both athletic groups had better diet quality and nutrition knowledge the $\mathbf{H_0}$ is accepted, no relationship exists between nutrition knowledge and diet quality.
- It was hypothesised that the macronutrient intake for carbohydrate and protein would be significantly different between the endurance and power groups. As measured by the 3-day diet record, no difference in macronutrient intake between the two athletic groups existed, therefore $\mathbf{H_0}$ is accepted.

To the author's knowledge, this is the first study to investigate the use of metabolic typing questionnaire in a research situation. The results identified 94.3% (n = 83) of

the participants as mixed types (type B), 2.3% (*n* = 2) as carbohydrate types (type A) and 3.4% (*n* = 3) as protein types (type C). Such an overwhelming dominance of one particular type has provided a number of questions about the application of the metabolic typing questionnaire. Firstly, are the questions within the metabolic typing questionnaire sufficient to distinguish between metabolic functions and subsequent metabolic type, secondly; if the questionnaire is accurate and 94.3% of the population are highlighted as a mixed type (thus should consume the same mixed diet) is the method of dietary individualisation relevant for healthy individuals, and thirdly; is the MTQ a valid tool for dietary individualisation prescription.

Due to the low number of individuals highlighted as either 'carbohydrate type' (type A) or 'protein type' (type C) statistical analysis could not be completed. Interestingly there is very little difference in the habitual dietary macronutrient intake of the 3 metabolic 'types' (Chapter 5.3.2), however the individuals identified as protein types habitually consumed less protein than the carbohydrate types (14% vs. 19%). Both Walcott and Fahey (2000) and Kristal and Haig (2002) state numerous case studies where the application of the metabolic typing diet cured chronic illness where traditional medicine had previously failed. However every case study example has one thing in common: all the patients suffered from a predisposed metabolic or health disorder and the subsequent dietary intervention prescribed significantly changed and improved the health condition of the patient. No case studies in either book detail when the application of the method was unsuccessful at improving health (of which it can be presumed many cases exist). In general the athletes ate a balanced mixed diet (57% CHO, 17% PRO and 26% FAT), as did the healthy control group (54% CHO, 16% PRO and 29% FAT), diet quality was adequate in both populations and the participants did not suffer from chronic or acute ill health. Taking this into account it can be suggested that if an individual is not suffering from any significant health condition and is eating a balanced diet, then the metabolic typing questionnaire is not sensitive enough to prescribe an individualised diet. Research is needed, in clinical populations, to investigate its efficacy in patients suffering from chronic ill health. Finally, a recent review article concluded that genotype-based personalised nutrition is not developed enough, or fully understood, to advise evidenced-based practice and further research is needed (Senekal, 2012). As such, if personalised nutrition is not understood at a

genotyping level, it is fair to assume that basing a diet on the outcome of a 65-item questionnaire is probably not sufficient to prescribe an individualised diet.

The present study is the first to evaluate the nutrition knowledge of UK track and field athletes relative to a non-athletic control group. The use of the valid and reliable questionnaire developed specifically for this population adds to the robustness of the results (Chapter 4). The results of the NKQA demonstrated that UK track and field athletes have greater nutrition knowledge than a non-athletic control group (60.4% vs. 48.6%). In a similar study in a cohort of elite Australian athletes, Spendlove et al. (2012) demonstrated no significant difference in nutrition knowledge between athletes (57.6%) and a non-athlete group (63.1%). Although the findings of this study are in disagreement to that of Spendlove et al. (2012) the nutrition knowledge scores of the athletic cohorts from both studies are very similar (60.4% and 57.6%). It is important to consider that both studies used a different questionnaire to assess nutrition knowledge, however the NKQA used in this study was validated using a dietetic control group, who scored 80.4% on the questionnaire, which is similar to the dietetic trained calibration group (86.2%) used by Spendlove et al. (2012). As such, it is fair to assume that both questionnaires are equally challenging. The non-athletic control group from Spendlove et al. (2012) scored significantly higher than the results observed in this study (63.1% vs. 48.6%), as such it may be assumed that the non-athletic Australian control population who participated for the study had a better grasp of nutrition knowledge than the control group used in this study (Spendlove et al., 2012).

A systematic review from Heaney et al. (2011) detailing research in nutrition knowledge assessment of athletes up to 2011, highlights an average nutrition knowledge score of 59 ± 11% (mean N = 79 ± 68) (excluding Douglas and Douglas (1984) who had 943 participants). It is important to consider that the range of validated questionnaires and level of athlete varies significantly through all these studies; however these results provided a general cross sectional overview into nutritional knowledge in athletes. Similar results can be concluded from this study with the mean NKQ score of 60.4 ± 16.3, thus the overall nutrition knowledge of UK track and field athletes are in line with previous literature.

The highest scoring sub-categories in the NKQA for the athletic group were fat (66.6%) and protein (66.0%), the carbohydrate (55.8%) and fat (54.7%) sub-sections produced the highest scores for the control group (Figure 5.3.7). The protein sub-section produced the greatest difference in scores KNQ score; the athletes scored 20.6% higher than the control group (66.0% vs. 45.4%). This is interesting as the importance of protein for exercise performance, recovery and growth and repair is well documented in the literature (Tipton et al., 2007, Jeukendrup, 2011, Burke et al., 2007, Phillips and Van Loon, 2011, Stellingwerff et al., 2011). Additionally, protein science is a frequent topic of discussion in athletic magazine publications and websites, potentially increasing the athletes' exposure to protein science and its role in athletic performance. In the 10 questions specific to whether a food item is high or low in protein the athletic group scored 85% correct response. The athletes also had a strong understanding of the role protein plays within the body, with 88% correct responses. However when questioned about recommended daily allowances and how much protein is in different food items the athletic group provided a correct response less than 25% of the time. This clearly demonstrated that athletes understand what protein is however more education is needed around protein quality and quantity.

As discussed in Chapter 2.1.4, it is understood that speed and power athletes have different dietary requirements to endurance athletes, with literature stating a greater protein need for speed and power athletes and increased carbohydrate demand for endurance athletes (Tipton et al., 2007, Jeukendrup, 2011, Stellingwerff et al., 2011). The results from the current study demonstrate no significant difference in macronutrient intake between the two athletic groups. A higher fat intake in the control group was the only significant difference observed in macronutrient intake (Figure 5.3.8). The DQI is not a reflection of macronutrient ratio, rather a focus on food quality and micronutrients (Haines et al., 1999). When assessing for diet quality both the endurance and power groups scored significantly ($p < 0.05$) higher in the DQI than the control group, an average score ~9% better (Figure 5.3.6) which corroborate the findings from Rash et al. (2008), who observed a mean DQI score of 76% in American collegiate track athletes.

No between group difference was observed in the carbohydrate sub-section (59.2% vs. 55.8%, NUT vs. NONUT). Interestingly a higher percentage of the control group

said they knew what glycemic index was (65.5% vs. 55.9%), but on the 8 subsequent questions referring to glycemic index (GI) both groups averaged 47%. However if the participant answered 'NO' when asked if they knew what glycemic index was, they were told to skip the remaining question on it, and of the participants who answered the GI questions the athletes actually scored higher (85% vs. 72%).

Relatively little data exists on current dietary habits of track and field athletes. In Japanese athletes, Sugiura et al. (1999) reported no difference in dietary intake between sprint and endurance athletes (protein; 15 ± 2% vs. 16 ± 2%, fat; 32 ± 3% vs. 34 ± 5% and carbohydrate; 54 ± 5% vs. 51 ± 6%). The results corroborate the findings from this study; no difference in dietary intake exists between speed and endurance track and field athletes. Sugiura et al. (1999) also reported adequate diet quality, as observed in this study. However it is important to contextualise and consider the comparison of these two studies as the cultures and habitual dietary intake between populations are vastly different (Finegold et al., 1974). It has not been possible to find further literature to compare dietary intake in different track and field disciplines.

This is the first study to investigate the relationship between dietary quality and nutrition knowledge of UK track and field athletes, demonstrating no relationship exists between nutrition knowledge and diet quality (Figure 5.3.11) Interestingly both athletic groups scored significantly higher on the NKQA (Figure 5.3.4) and DQI (Figure 5.3.6) compared to the control group, but neither NKQA nor DQI were a predictor of the other. It may be concluded that athletes have better nutrition knowledge and diet quality; however no associations can be drawn between the two. To date the literature is equivocal, recently Heaney et al. (2011) published a meta analysis partly on nutrition knowledge and dietary intake. Out of the nine studies published, five reported a weak positive association between nutrition knowledge and better dietary intake ($r < 0.44$), the remaining four studies were unable to link nutrition knowledge to diet quality. Interestingly three of the studies that demonstrated a positive relationship used non-validated sport specific questions to assess diet quality, as such caution should be taken when interpreting the results (Douglas and Douglas, 1984, Harrison et al., 1991, Hamilton et al., 1994). Including the results from the current study, 50% of the research in this area has demonstrated a weak positive relationship in nutrition knowledge and

dietary quality and 50% demonstrated no relationships. Finally, most studies in athletes with a robust design have demonstrated no relationship.

The findings from the diet logs, NKQA and DQI suggest that athletes have sound fundamental nutrition knowledge and consume a high quality balanced diet. However dietary intake is not tailored to the specific demands of their sporting discipline. The NKQA results demonstrate athletes generally understand what macronutrients are, however understanding of the amount of each macronutrient present in food items and the current dietary recommendations for athletes are not as well grasped. This may explain why no difference in dietary intake is observed between power and endurance athletes.

5.5 Conclusion.

In conclusion, the data from the current study suggests a 65-item metabolic typing questionnaire is not sensitive enough to prescribe individualise diets for healthy or athletic populations and no association exists between metabolic type and habitual dietary intake. Additionally, UK track and field athletes possess greater nutrition knowledge than a population matched, non-athletic control and the diet quality of the athletes is greater than that of the control group. However no association between diet quality and nutrition knowledge was present.

5.6 Limitations.

The limitations of the experimental research Chapter are:

- Dietary intake was measured using a 3-day food diary using estimated household measures. As discussed in Chapter 2.4 dietary assessment in a free-living population has limitations and may under-predict intake.
- Diet quality index score is based on a snapshot of dietary intake which may have been skewed by the study. A longitudinal analysis of dietary intake may have provided a more comprehensive analysis of dietary intake, however this increases participant burden and would compromise the study.

- Metabolic function was not directly assessed in this study thus further study is needed to draw strong robust conclusions around the relationship between metabolic type and metabolic function in athletes.

5.7 Future research for experimental Chapter.

The results from this experimental Chapter have provided evidence suggesting that the metabolic typing questionnaire is not refined enough to differentiate sporting classification and subsequently prescribe a diet to meet individual requirements. The theory of metabolic typing is based on three diets: high carbohydrate, mixed and high protein diet. The fundamental impact of such diets at a cellular level are yet to be fully understood, thus the remaining Chapters in this thesis are to explore the impact of high protein and high carbohydrate diets on cellular adaptation at rest and with exercise training.

6 THE IMPACT OF A HIGH PROTEIN DIET ON CELLULAR METABOLIC ADAPTATION, BODY COMPOSITION AND RESTING METABOLIC RATE.

Abstract:

Background: Mitochondrial quantity and density are associated with increased oxidative metabolism. It has been demonstrated that a high fat/low carbohydrate (HF/LC) hypocaloric diet can up-regulate transcriptional markers of mitochondrial biogenesis. The impact *in vivo* of a high protein/low carbohydrate (HP/LC) diet has on these markers is still relatively unknown. The aims of the study were to investigate the impact of acute (7-day) dietary manipulation on transcriptional markers of mitochondrial biogenesis, body composition and resting metabolic rate. **Method:** Forty-five healthy male participants were randomly assigned one of four dietary conditions: energy matched high protein (PRO-EM), hypocaloric high protein (PRO-ER), energy matched high carbohydrate (CHO-EM) or hypocaloric high carbohydrate (CHO-ER). The macronutrient ratio of the high protein diet was 40:30:30 % (PRO:CHO:FAT), the high carbohydrate groups were on 10:60:30 %. Energy intake for the hypocaloric diets were calculated to match resting metabolic rate. Participants visited the laboratory on 3 occasions each separated by 7 days. On each visit body composition, resting metabolic rate and a muscle biopsy from the vastus lateralis was collected. Prior to visit 1 and 2 habitual diet was consumed, between visit 2 and 3 the intervention diet was consumed. **Results:** In the PRO-ER group AMPK, PGC-1α, SIRT1 and SIRT3 mRNA expression were significantly increased ($p < 0.05$) post intervention. Transcriptional markers were not affected in any of the other groups. Despite ~30 % reduction in calorie intake no difference in lean mass (LM) loss was observed between the PRO-ER and CHO-EM groups. **Conclusion:** The results from this study demonstrated that a 7-day a high protein hypocaloric diet resulted in increased mRNA transcriptional markers of mitochondrial biogenesis at rest, also increased protein intake helped to maintain LM mass in a hypocaloric state.

6.1 Introduction

Mitochondria are responsible for energy production via fatty-acid oxidation, β oxidation, Krebs cycle, and oxidative phosphorylation. Mitochondria quantity and density has been linked with increased endurance performance (Psilander et al., 2013), reduction in type 2 diabetes, increased resisting metabolic rate and improved insulin sensitivity (Lee et al., 1998, De Feyter et al., 2008, Patti et al., 2003, Petersen et al., 2004, Mootha et al., 2003). A seminal paper from Puigserver et al. (1998) first described peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), in the subsequent years PGC-1α had been identified as playing a key role in the regulation and co-activation of mitochondrial biogenesis (Liang and Ward, 2006).

Both AMP-activated protein kinase (AMPK) and silent information regulator T1 (SIRT1) mediated deacetylation (Gurd et al., 2009) have been demonstrated to regulate the expression of PGC-1α in human muscle (Ruderman et al., 2010). A substantial body of evidence suggests that SIRT1 expression responds to decreases in nutrient availability (Nemoto et al., 2005) and increases in energy expenditure (Suwa et al., 2008). Similarly AMPK is stimulated by cellular stress that causes a depletion of adenosine triphosphate (ATP) and elevation of AMP, such as calorie restriction (Itani et al., 2003), hypoglycemia (Itani et al., 2003) exercise (Fujii et al., 2000) and muscular contraction (Hutber et al., 1997). Subsequently increases in PGC-1α expression can be observed in direct response to such stressors (Jager et al., 2007). Acute hypocaloric HF/LC diet (50% fat, 30% carbohydrate and 20%, protein) significantly increases AMPK phosphorylation and PGC-1α deacetylation, however no change was observed in a hypocaloric LF/HC diet (20:60:20 %) (Draznin et al., 2012), suggesting increased dietary carbohydrate availability prevents the activation of the AMPK-SIRT1 axis in skeletal muscle that would otherwise be activated by a low calorie diet.

The preservation of lean mass is important for the maintenance of quality of life (Janssen et al., 2002), it is generally accepted that weight loss strategies which preserve LM are preferential to those that results in skeletal muscle atrophy (Stiegler and Cunliffe, 2006). During hypocaloric diet-induced weight loss approximately 20 –

30% of mass lost is lean mass (LM) (Areta et al., 2014), increasing dietary protein is one method which has been demonstrated to attenuate skeletal muscle atrophy in a hypocaloric state (Stuart et al., 1990). The manipulation of carbohydrate intake as a regulator of weight maintenance/loss is well documented demonstrating improved lipid profile and fat oxidation (Boden et al., 2005, Hussain et al., 2012). However, the majority of literature manipulates dietary fat – protein remains constant. A small number of metabolic perturbation murine studies have manipulated protein intake and it has been shown that a high protein intake (35 % protein) increases PGC-1α expression relative to a control diet (15% protein) (Nakazato and Song, 2008). Furthermore in periods of energy deficiency it has been demonstrated that resting metabolic rate can be reduced (Friedlander et al., 2005), and in overweight and obese individuals attempting to lose weight, dietary restriction can result in decreases in muscle mass which may down-regulate the metabolic process, compromising healthy weight management (Ravussin et al., 1988, Stein et al., 1991). Increased protein intake in periods of energy restriction may support the maintenance of metabolic rate (Carbone et al., 2012).

In this field most research focuses on increasing dietary fat to restrict carbohydrate intake. However it is well documented that an increased protein intake can attenuate LM loss and may be a preferable choice during weight loss. The impact high protein low carbohydrate diets have on transcriptional markers of mitochondrial biogenesis is not fully explored. This study was designed to investigate the impact of a high protein low carbohydrate diet independently and with calorie restriction on metabolic adaptation, body composition and RMR.

6.1.1 Aims and Objectives.

The primary aim of this piece of research was to see if manipulating dietary intake for a 7-day period provided enough metabolic stress to cause a change in cellular transcriptional response, body composition and resting metabolic rate.

The following hypotheses have been made for this body of research:

i) (**H_1**) The diet that provides the greatest metabolic stress (high protein hypocaloric diet) will elicit the greatest mRNA transcriptional changes in skeletal muscle.
   (**H_0**) No difference in skeletal muscle mRNA transcriptional response will exist between groups.
ii) (**H_2**) When matched for energy intake, diets with a high protein intake will promote lean mass maintanence to a greater extent than the equivalent high carbohydrate groups.
   (**H_0**) No difference in body composition will be observed between the groups matched for energy intake.
iii) (**H_3**) The high protein energy matched group will result in an increase in resting metabolic rate.
   (**H_0**) No change in resting metabolic rate will occur in any of the groups subsequent to the 7-day dietary intervention.

6.2 Materials and Methods.

6.2.1 Participants.

Forty-eight healthy males volunteered to participate in the study with 45 completing (mean ± SD: age 26.0 ± 5.1 years; body mass 74.9 ± 10.2 kg; height 179.5 ± 5.9 cm). One participant was removed due to non-dietary adherence, a second was removed as inclusion criteria were not met and a third did not complete. Participants were initially screened against pre-determined criteria to ensure they met the inclusion criteria and were free from any medical condition that would preclude participation in the study. Participants could not be following a restrictive dietary regime (vegetarian, vegan) or suffer from any food allergies/intolerances. Participants could not be participating in physical activity > 2 times per week and could not be consuming or have consumed any dietary or protein supplements in the previous 2 weeks. The experimental procedures and potential risks associated with the study were explained and the participants gave written informed consent prior to participation. None of the participants had a history of any neurological disease or musculoskeletal abnormality. The University of Hertfordshire School of Life and Medical Sciences ethics committee approved the study, protocol number LMS/PG/UH/00196.

6.2.2 Study Protocol.

In a randomised repeated-measures parallel group study design, the participants were assigned to one of four groups: energy matched high protein (PRO-EM, *n* = 11), energy restricted high protein (PRO-ER, *n* = 12), energy matched high carbohydrate (CHO-EM, *n* = 12) or energy restricted high carbohydrate (CHO-ER, *n* = 10) (Table 6.2.1). The participants attended the laboratory on three occasions, each separated by 7-days. Before visit 1 (baseline) and visit 2 (pre) all participants remained on their habitual diet, between visits 2 and visit 3 (post) the participants consumed the randomly assigned intervention diet. All experimental procedures were completed in the same order at the same time of the day. The baseline and pre assessments were used as a control to demonstrate stability of the measures assessed, where no differences were observed between these measures the pre measures were used for analysis.

Table 6.2.1**.** Prescribed macronutrient breakdown for each group.

	Prescribed calorific intake	Carbohydrate (%)	Protein (%)	Fat (%)
PRO-EM	Matched to estimated daily energy requirements	30	40	30
PRO-ER	Restricted to resting metabolic rate	30	40	30
CHO-EM	Matched to estimated daily energy requirements	60	10	30
CHO-ER	Restricted to resting metabolic rate	60	10	30

(% = percentage of total daily calorific intake)

6.2.3 Experimental protocol.

Following an overnight fast the participants arrived at their allocated timeslot for each testing visit (start time range: 07:30 – 09:30 am). The participants were requested to arrive at the laboratory using the same mode of transport. Morning physical activity was not permitted. Prior to the pre and baseline visits the participants completed a standardised 4-day food dairy. The food diary was visually assessed for accuracy by

the investigator and further detail was requested if required. An assessment of body composition (DXA scan) was then undertaken, followed by the measurement of resting metabolic rate. Finally a micro-muscle biopsy was collected (Figure 6.2.1).

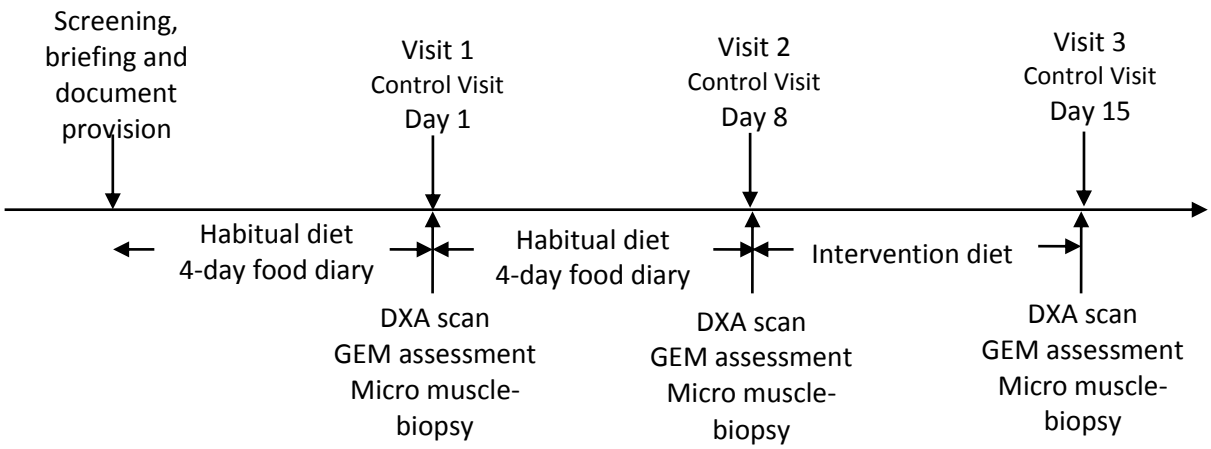


Figure 6.2.1. Schematic of experimental protocol.

Inclusion criteria: Healthy males aged between 18 – 35 years with no identified chronic illness and currently free from cold, flu or any musculoskeletal injury.

Exclusion criteria: Outside the range of 18 – 35 years, female, suffering from heart or chronic illness. If currently following a restrictive dietary regime which excludes any food groups (e.g. Vegan, Vegetarian), or if the participant is aware of any food allergies. If consuming any nutrition supplements (or unwilling to abstain from taking during the study), or on medication (beta-blockers, NSAID's or recreational drugs).

6.2.4 Measurement of habitual dietary intake.

For the method used to calculate habitual dietary intake refer to Chapter 3.2. Participants were requested to record intake on 3-week days and 1 weekend day each week, a total of 8-days dietary intake was collected over the 2 week prior visit 1 and visit 2.

6.2.5 Body composition assessment: Dual energy x-ray absorptiometry (DXA) scan.

A DXA scanner was used to measure body composition. Please refer to Chapter 3.6 for details regarding the processes complete to measure body composition using the DXA scanner.

6.2.6 Resting metabolic rate (RMR).

RMR was measured using indirect calorimetry (coefficient of variation = 1.48 %). All assessments were completed in a temperature controlled environmental chamber (temperature set to 24°C). The participants arrived at the laboratory in the morning in an overnight fasted state. The participants were requested to expel as little energy as possible prior to arrival. The participant lay in a supine position on a couch and a large towel was placed over their body to maintain comfort. Once comfortable a metabolic hood (Gas Exchange Measurement (GEM), GEMNutrition Ltd, UK) was placed over the participant's head. The participant was instructed to relax, breathe normally and not to move or fall asleep (Figure 6.2.2). The GEM was set to collect respiratory bins every 60 seconds for 20 minutes; the data analysed was from the final 5 minutes of collection. Throughout the test the participant was observed through a window to check for adherence. RMR was calculated using the modified Weir equation (Weir, 1949):

$$\text{“RMR} = 1.44 \times (3.9 \times \dot{V}O_2 + 1.1 \times \dot{V}CO_2)\text{”}$$

(The modification of the equation negates the effect of nitrogen excretion, it is generally accepted that the errors associated with collecting reliable nitrogen samples are greater than the small error introduced by not including it)

Figure 6.2.2. Participant undergoing a resting metabolic rate test using the GEM.

Respiratory exchange ratio was calculated to assess for changes in resting substrate utilisation by dividing the $\dot{V}CO_2$ value ($L \cdot min^{-1}$) by the $\dot{V}O_2$ value ($L \cdot min^{-1}$) from each minute bin collected on the GEM.

6.2.7 Micro muscle biopsy.

For the micro muscle-biopsy procedure used, please refer to Chapter 3.4 in the general methods section.

6.2.8 Dietary prescription.

For the two energy restricted (ER) groups the participants were provided an intervention diet with an energy intake that met RMR. RMR was calculated from the GEM result attained from Visit 1. For the energy matched (EM) groups, energy intake was calculated according the World-Health-Organization (2003) lifestyle dependent physical activity multiplier:

"EI = PAF x RMR"

EI = Energy intake, PAF = physical activity factor, RMR = resting metabolic rate

RMR was attained from the GEM result on visit 1, and PAF was selected following a series of lifestyle questions concerning daily activity levels, type of work and exercise activity (Table 6.2.2).

Table 6.2.2. Lifestyle dependent physical activity multiplier used to calculate energy intake.

Activity level	Calculation
Low	1.53 x RMR
Moderate	1.77 x RMR
Active	2.25 x RMR

(World-Health-Organization, 2003)

The intervention diets (Table 6.2.3) were calculated by the experimental team and delivered to the participant's door by Soulmate food. Refer to Section 3.3 in the general methods for description of the process.

Table 6.2.3. Prescribed daily calorie intake and macronutrient breakdown for each group throughout the 7-day intervention.

Group	Dietary intake (kcal•day^{-1})	CHO (•day^{-1})		PRO (•day^{-1})		FAT (•day^{-1})	
		g	g•kg^{-1}	g	g•kg^{-1}	g	g•kg^{-1}
PRO-EM	2828 ± 331	217 ± 26	3.0 ± 0.5	289 ± 34	4.0 ± 0.7	96 ± 11	1.3 ± 0.2
PRO-ER	1876 ± 116	143 ± 9	1.9 ± 0.3	191 ± 12	2.5 ± 0.4	64 ± 4	0.8 ± 0.1
CHO-EM	2881 ± 213	444 ± 33	6.1 ± 0.6	75 ± 6	1.0 ± 0.1	97 ± 7	1.3 ± 0.1
CHO-ER	1735 ± 246	267 ± 37	3.7 ± 0.7	45 ± 6	0.6 ± 0.1	59 ± 8	0.8 ± 0.2

(Mean ± SD). PRO-EM = high protein energy matched diet, PRO-ER = high protein energy restricted diet, CHO-EM = high carbohydrate energy matched diet, CHO-ER = high carbohydrate energy restricted diet.

6.2.9 Quantitative PCR (qPCR).

The analysis of the gene expression in the muscle was quantified using qPCR. Refer to Chapter 3.5 in the general methods for a detailed description of the RNA isolation, reverse transcription, qPCR analysis and application efficiency techniques used in this experimental Chapter.

6.2.10 Data analysis of the qPCR assays

The muscle biopsy qPCR raw Cp values were exported and normalised to the housekeeper gene (GAPDH) using an analysis of covariance method. Relative fold change in expression levels over baseline (visit 2) was then calculated using $2^{-\Delta\Delta C_T}$ method.

6.2.11 Statistical analysis.

Kolmogorov-Smirnov tests of normality to assess the null hypothesis that data for within-subjects analysis was normally distributed. However it should be noted that the ANOVA procedure is robust to slight violations of normality within a dataset (Field, 2013). Mauchly's sphericity test was used to assess the assumption of sphericity within repeated-measures effects. Unless stated otherwise Mauchly's test was insignificant ($p > 0.05$), therefore the assumption of sphericity was accepted

The muscle biopsy qPCR analysis raw Cp values were normalised to the housekeeper gene (GAPDH) using an analysis of covariance method, before relative fold change over TP1 (baseline) were calculated using $2^{-\Delta\Delta CT}$.

mRNA data were analysed using a two factorial mixed design ANOVA (4 groups, 2 time points) with dietary intake (between) and time point (within) as the main variables. Where a main time effect was observed a within group paired sample assessments was used to identify where the time effect was. A Bonferroni post hoc test was used to identify the location of significant interactions between groups (dietary intake). Visit one (baseline) and visit two (pre) were used as control trials; a paired sample t-test was run to determine a difference between the control trials. Where difference in

baseline-pre was observed a mean of the two values was used for post comparison (which is stated in the text), where no difference was observed, the pre value was used for comparison. Pre-post intervention time point change was analysed using a paired sample t-test.

Statistical significance was set at $p \leq 0.05$. Statistical analysis was conducted using the statistical package for the social science software program (SPSS; version 22, IBM, Armonk, NY). All data are presented mean ± SEM unless specified.

6.3 Results

6.3.1 Habitual dietary intake

No main effects were observed for habitual total calorie intake or macronutrient breakdown (Table 6.3.1). The mean daily calorific intake across all groups was 2,340 ± 473 kcal•day^{-1} (range 2,192 ± 574 – 2423 ± 330 kcal•day^{-1})

Table 6.3.1. Average daily habitual calorific intake and macronutrient breakdown for each group prior to intervention diet.

(mean ± SD and percentage (%) of total energy intake).

	Mean daily calorie intake (kcal•day-^{1})	CHO		PRO		FAT	
		g•day^{-1}	%	g•day^{-1}	%	g•day^{-1}	%
PRO-EM	2192 ± 173	232 ± 22	42.4	95 ± 6	17.2	86 ± 6	35.2
PRO-ER	2391 ± 133	267 ± 16	44.7	100 ± 8	16.7	93 ± 5	35.0
CHO-EM	2355 ± 152	274 ± 23	46.6	103 ± 9	17.5	89 ± 8	33.8
CHO-ER	2407 ± 107	307 ± 16	51.7	97 ± 7	16.0	84 ± 5	31.4

A total of 8-days dietary intake was collected over the 2 week prior visit 1 and visit 2; participants were requested to record intake on 3 week days and 1 weekend day each week using the estimated household measures method. (CHO = carbohydrate, PRO = protein, FAT = fat). % = percentage of dietary intake

The PRO-ER groups lost significant greater BM relative to both CHO-EM and PRO-EM, the same interactions were observed with the CHO-ER group.

6.3.2 Gene expression

No significant difference was observed between TP1 and TP2 in any of the genes, thus it can be assumed that any post intervention changes observed were due to the intervention rather than the internal variability of the measure.

The output of statistical analysis demonstrated no group x time interaction in any of the genes (PGC-1α: $f = 0.900$, $p = 0.494$, SIRT1: $f = 1.022$, $p = 0.447$, AMPK: $f = 2.827$, $p = 0.29$, SIRT3: $f = 2.843$, $p = 0.128$ and PPAR: $f = 2.068$, $p = 0.145$). However a time effect was observed in some of the genes which warranted further exploration to identify where the time difference existed (PGC-1α: $f = 6.020$, $p = 0.048$, SIRT1: $f = 6.297$, $p = 0.037$, AMPK; $f = 15.263$, $p = 0.005$ and SIRT3: $f = 9.628$, $p = 0.015$). Within the PRO-ER group a significant pre-post intervention time point difference was observed in PGC-1α (fold increase = 1.27, $p = 0.0402$), AMPK (fold increase = 2.09, $p = 0.027$), SIRT1 (fold increase = 1.50, $p = 0.026$) and SIRT3 (fold increase = 1.19, $p = 0.010$) mRNA expression. No time point gene expression changes were observed in any other dietary group (Figure 6.3.1 A-E), or in the expression of PPAR mRNA.

A significant change in LM was observed post intervention as were significant group differences. Post intervention, 3 of the groups lost LM ($p < 0.05$) (Figure 6.3.2 A), the CHO-ER losing the greatest amount (-1.26 ± 0.14 kg), the PRO-ER losing -0.82 ± 0.3 kg and CHO-EM losing -0.53 ± 0.19 kg, respectively. LM was maintained in the PRO-EM group (-0.21 ± 0.17 kg). The CHO-ER group lost significantly more LM than both the PRO-EM and CHO-EM groups ($p < 0.05$), no difference in LM was observed between the PRO-ER and CHO-EM groups.

The PRO-ER and CHO-ER groups lost significantly greater ($p < 0.05$) BM than both CHO-EM and PRO-EM (Figure 6.3.2 C). No difference was observed between groups matched for energy intake. All groups exhibited a significant loss in FM ($p < 0.01$) post 7-days dietary intervention (Figure 6.3.2 B). The PRO-ER lost the greatest amount of FM (-0.99 ± 0.11 kg) and the CHO-EM group lost the least (-0.50 ± 0.14 kg). The difference between these two groups was the only significant group difference observed ($p = 0.015$).

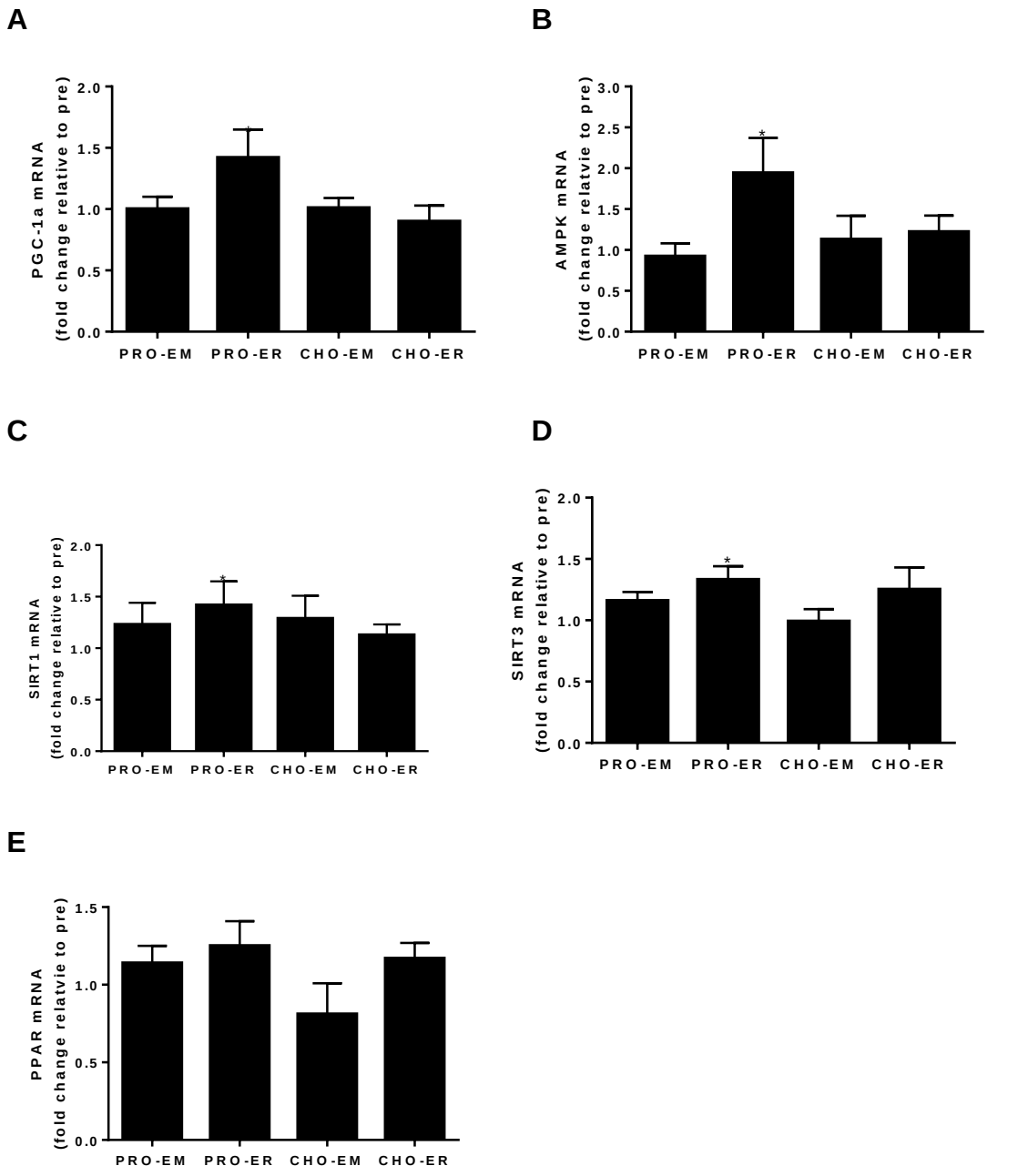


Figure 6.3.1. Effect of feeding 7-days diet intervention on mRNA expression at rest.

*Effect of 7-days high protein energy matched (PRO-EM), high protein energy restricted (PRO-ER), high carbohydrate energy matched (CHO-EM) or high carbohydrate energy restricted (CHO-ER) diet in healthy lean individuals on PCG-1α (**A**), AMPK (**B**), SIRT1 (**C**), SIRT3 (**D**) and PPAR (**E**) mRNA expression. Muscle samples were obtained following an overnight fast prior to the intervention and repeated again following 7-days dietary intervention (Post). Values are expressed as fold change (pre to post) after normalization to the reference gene (GAPDH) and are reported as the mean ± SE. For abbreviations of genes see Table 1. * = significant pre-post difference within group, $p < 0.05$.*

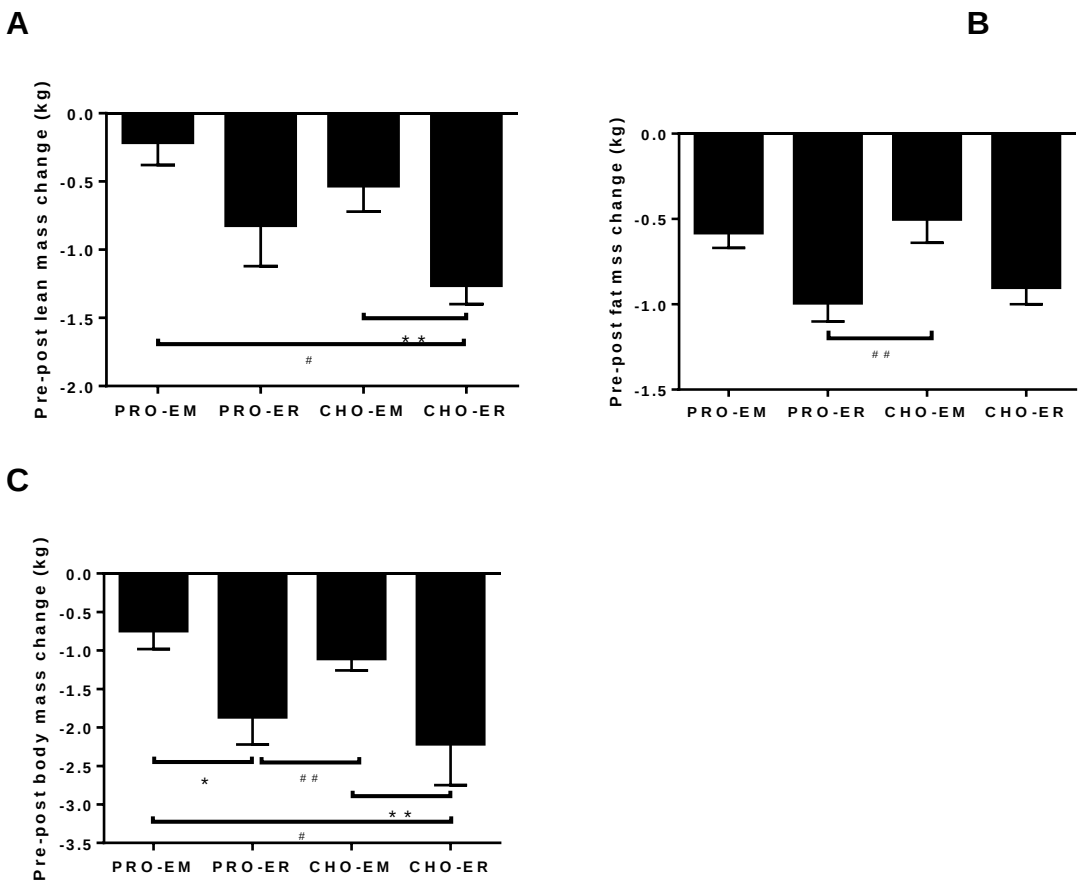


Figure 6.3.2**.** Effect of feeding 7-days diet intervention on body composition at rest.

*Effect of feeding 7-days of either high protein energy matched (PRO-EM), high protein energy restricted (PRO-ER), high carbohydrate energy matched (CHO-EM) or a high carbohydrate energy restricted (CHO-ER) diet in healthy lean individuals on body composition. Pre-post lean mass change (kg) (**A**), Pre-post fat mass change (kg) (**B**). Pre-post body mass change (kg) (**C**). The data presented as mean ± SEM; * denotes significant difference between PRO-EM and PRO-ER, ** denotes significant difference between CHO-EM and CHO-ER, # denotes significant difference between PRO-EM and CHO-ER and ## denotes significant difference between PRO-ER and CHO-EM. (p < 0.05).*

A post intervention reduction in BM was observed in all groups ($p < 0.01$, Figure 6.3.2 C). The PRO-EM group lost the least BM (-0.74 ± 0.24 kg) whereas the CHO-ER lost the greatest (-2.21 ± 0.17 kg). Significant post intervention group differences were observed ($p < 0.01$), this was not seen in groups matched for energy intake, however both ER groups lost significantly more BM than EM groups.

6.3.3 Resting metabolic rate and body composition response to dietary manipulation

No time or group interaction effects were observed in RMR (Table 6.3.2). Mean RMR across all groups pre was 1910 ± 180 kcal vs. 1894 ± 184 post intervention.

Table 6.3.2. Resting metabolic rate (RMR) (kcal•day^{-1}) pre and post 7-day dietary intervention .

	RMR PRE (kcal•day^{-1})	RMR POST (kcal•day^{-1})
PRO-EM	1916 ± 73	1910 ± 70
PRO-ER	1904 ± 37	1950 ± 54
CHO-EM	1972 ± 38	1946 ± 44
CHO-ER	1848 ± 69	1768 ± 52

PRO-EM = high protein energy matched diet, PRO-ER = high protein energy restricted diet, CHO-EM = high carbohydrate energy matched diet, CHO-ER = high carbohydrate energy restricted diet (Mean ± SEM).

A significant time point interaction was observed in respiratory exchange ratio (f = 13.811, p = 0.005). No difference was observed in either of the energy-matched groups, however RER significantly reduced subsequent to the intervention diet in both the PRO-ER (p = 0.001) and the CHO-ER (p = 0.008) groups (Figure 6.3.3).

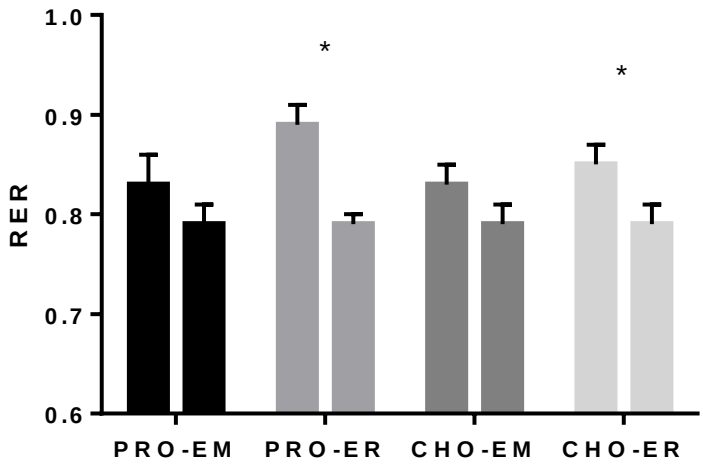


Figure 6.3.3. Respiratory exchange ratio (RER) attained from the GEM test pre and post dietary intervention (mean ± SEM)

** Denotes significantly difference from pre (p < 0.01)*

6.4 Discussion

With regards to the research hypothesis the following can be concluded:

i) It was hypothesised that a high protein hypocaloric diet would elicit the greatest mRNA transcriptional changes in skeletal muscle. Subsequently H_0 was accepted as no group difference in mRNA expression was observed. As measured by the mRNA attained from the muscle-biopsies no group x time difference were observed in any of the transcriptional mRNA markers of mitochondrial biogenesis. However a pre-post time point difference was observed in four mRNA markers in the PRO-ER group.

ii) It was hypothesised that no difference in body composition would be observed between the groups matched for energy intake. In rejection H_0 body composition was impacted by the intervention. As measured by the DXA scan, significant post intervention between group differences in body mass, fat mass or lean mass were observed

iii) It was hypothesised that the high protein energy matched group would result in an increase in resting metabolic rate. In support of H_0, as measured by the GEM, no change in RMR was observed in either group or time point.

This is the first acute dietary intervention study in healthy sedentary males to demonstrate that increased dietary protein intake in a hypocaloric state positively increased mRNA expression of PGC-1α, AMPK and SIRT1 + 3.

In a myoblast cell line it has previously been demonstrated that both calorie and carbohydrate restriction independently increase transcriptional markers of mitochondrial biogenesis. Following 36 hours incubation in a low glucose solution (5 mM) a > 2.5 fold increase in PGC-1α transcriptional activity was observed relative to a high glucose solution (25 mM), additionally the same authors also demonstrated that following a 20-hour fast, relative PGC-1α mRNA levels were increased > 2 fold, suggesting positive activity *in vitro* of energy and glucose restriction independently (Canto et al., 2010). However, *in vivo* results from the current study demonstrated no change in mRNA expression in the PRO-EM (carbohydrate restriction) or CHO-ER (energy restriction) groups, suggesting that acute (7-day) carbohydrate restriction and energy restriction independently do not exert sufficient metabolic stress to elicit metabolic changes at an mRNA level. Furthermore the results from this study showed that subsequent to a continuous, hypocaloric high protein diet low carbohydrate (40% protein (~ 2.5 $g \cdot kg^{-1} \cdot day^{1}$) and ~33 % energy deficit) a small, but significant, increase in resting mRNA expression of AMPK, SIRT1, SIRT3 and PGC-1α in the vastus lateralis muscle was observed. It is important to recognise that no group difference were observed in this study, however a non-significant trend towards a group difference in AMPK (p = 0.056) and PGC-1α (p = 0.063) gene expression was observed, which suggests the study may have been slightly under powered. The outputs of a post-hoc power calculation suggests an effect size of 1.12 and sample size of each group being n = 18 which corroborates this. These results imply that, in contrast to independent restriction, the combination of energy and carbohydrate restriction may provided sufficient metabolic stress to cause skeletal muscle adaptation in order to cope with the restricted energy availability and meet energy demands.

These results are corroborated by similar studies investigating the impact of high fat feedings on mitochondrial biogenesis (Canto et al., 2010, Draznin et al., 2012). It was shown that five days on a hypocaloric HF/LC diet positively increased phosphorylation of AMPK and deacetylation of PGC-1α, whereas the hypocaloric LF/HC diet negatively

reduced the phosphorylation of AMPK and deacetylation of PGC-1α (Draznin et al., 2012). These results, along with the findings from the current study suggest that high carbohydrate intake prevents the activation of the AMPK-SIRT1 axis that is otherwise observed in a hypocaloric state. Furthermore, and importantly the method of carbohydrate restriction does not seem important, increasing both dietary fat and/or protein result in similar up-regulation of metabolic markers of mitochondrial biogenesis.

It is considered that weight loss strategies which result in maintenance of LM and promote greater FM loss are preferential (Patti et al., 2003). It has previously been demonstrated that ~70 % of weight lost, induced by a hypocaloric diet, is attributed to FM and 30 % from LM (Weinheimer et al., 2010). Furthermore Layman et al. (2003). reported increased FM loss and improved maintenance of lean mass in obese women with increased protein intake (1.6 $g \cdot kg^{-1} \cdot day^{-1}$ vs. 0.8 $g \cdot kg^{-1} \cdot day^{-1}$). Within a healthy non-obese male population the results from this study demonstrate similar findings. Although there was no difference in body composition changes between energy-matched groups, some further considerations can be drawn. When matched for macronutrient ratio, but manipulating energy intake the CHO-ER group lost significantly more LM than the CHO-EM group, however no difference in LM loss was observed between the PRO-ER and PRO-EM. This suggests that when restricting energy intake but maintaining macronutrient ratio a high protein low carbohydrate diet provides a sparing effect on lean mass loss relative to a high carbohydrate energy restricted diet. Similarly to the mRNA data, visually the body composition data suggests a group difference in energy matched groups may exist, but the study may not have powered enough to identify. The post-hoc power calculation for body composition suggests an effect size of 0.95 and a sample size of n = 26 per group. When looking as the four groups together the PRO-ER group had a higher protein intake relative to the CHO-EM group (2.5 $g \cdot kg^{-1} \cdot day^{-1}$ vs. 1.0 $g \cdot kg^{-1} \cdot day^{-1}$) and although the calorie intake of the PRO-ER group was ~33 % less than that of the CHO-EM group (total deficit of ~7,000 kcal over the 7-day intervention) no change in LM was observed between groups, further highlighting the key role for protein in LM maintenance. The mechanism of this is still not fully defined. The dietary protein intake of the CHO-EM group was greater than the current reference nutrient intake in the UK for adults of 0.75 $g \cdot kg^{-1} \cdot day^{-1}$ (Foundation, 2015), however it has been demonstrated

the proteolysis of skeletal muscle is suppressed when consuming ≥ 1.5 g•kg^{-1}•day^{-1} of protein (Piatti et al., 1994). The PRO-ER group consumed 2.5 g•kg^{-1}•day^{-1} of protein, significantly above this threshold.

With regards to weight loss, although non-significant, the results suggest a number of trends which warrant further exploration. Suggesting that when consuming a hypocaloric diet dietary protein intake could positively impact LM and FM loss. When looking and components of BM lost while following the continuous hypocaloric diet (~33% energy deficit) both groups (high protein, low carbohydrate and high carbohydrate) lost a significant amount of LM, approximately 44% of total BM loss in the PRO-ER group was attributed to LM loss compared to 56% in the CHO-ER group. This was coupled with a greater percentage of FM loss in the PRO-ER group (56% vs. 44% respectively). As such, it may be assumed that the increased dietary protein intake in the PRO-ER group (2.5 g•kg^{-1} vs. 0.6 g•kg^{-1}) attenuated LM loss by ~12 % relative to a hypocaloric diet low in protein. A recent meta-analysis (Wycherley et al., 2012) investigating the role of dietary protein intake and long term body composition changes (12 ± 9 weeks) concluded higher protein diets led to greater BM loss (-0.79 kg), FM loss (-0.87 kg) and preserved more LM (+0.43 kg) relative to lower protein diets. Although over a shorter period, the body composition results from the hypocaloric groups observed in this study followed the same trends with -0.35 kg BM, -0.09 kg FM and +0.44 kg LM body composition changes observed. It has been demonstrated that a positive curvilinear (r^2 = 0.92) relationship exists between LM and RMR (Weinsier et al., 1992), and RMR is known to decline during prolonged (> 9 months) periods of energy restriction (Heshka et al., 1990). The thermic effect of macronutrients should also be considered, protein has a greater thermic effect compared with the equivalent energy intake of fat or carbohydrate (Malik and Hu, 2007). However the acute nature of the intervention was not long enough the elicit change in RMR and no time point difference or group interaction was observed in RMR, thus it may be assumed that the duration of the study was not long enough to elicit changes in RMR, as such metabolic rate is not impacted by such a short dietary intervention.

A marked reduction in RER post intervention was observed in both ER groups; however caution should be taken when drawing strong conclusions from this data. Pre

intervention both ER groups had a higher RER (PRO-ER = 0.89, CHO-ER = 0.85, PRO-EM = 0.83, CHO-EM = 0.83) and post intervention all groups had an RER of 0.79. Thus a greater relative change was observed pre-post intervention in the ER groups, but no group interaction was apparent. In mice and subsequent to 8 weeks on a hypocaloric diet a significant reduction in RER was observed compared to relative to energy matched diets (Solon-Biet et al., 2015). Similar to the results from this study, RER was reduced to the same level in the hypocaloric group's independent of dietary protein intake (5% vs. 33% vs. 60%). Solon-Biet et al. (2015) also reported a significant reduction in RER in the eucaloric high protein group relative to the eucaloric high protein diet, however this was not to the amplitude of the any of the hypocaloric diets. This suggests that in an eucaloric state restricted carbohydrate (increased protein intake) results in a reduction in RER, however calorie restriction has a greater metabolic impact, independent on macronutrient ratio.

Interestingly this study raises further considerations for dietary prescription studies and methods used to measure energy intake. The mean calorie intake calculated from 2 x 4 day food diaries for each participant in the PRO-EM and CHO-EM groups were 2,192 ± 574 kcal•day^{-1} and 2,355 ± 527 kcal•day^{-1}, respectively. However, mean calculated energy intake from RMR X Activity Factor (AF) were 2,828 ± 331 kcal•day^{-1} and 2,881 ± 213 kcal•day^{-1}, a difference of 636 and 526 kcal•day^{-1}, respectively. Despite meticulous effort to match the dietary intake of the PRO-EM and CHO-EM to energy expenditure the results demonstrated a small reduction in BM in both group (0.74 kg and 1.1 kg respectively), as such dietary prescription was slightly underestimated. Despite this, habitual diet, as recorded using estimated household measures, under predicted energy intake calculated using RMR x PA by ~20%, a similar figure to previous findings in athletes (Magkos and Yannakoulia, 2003) (~28 %) and sedentary participants (Stockley, 1985) (~20%).

6.5 Conclusions

Mitochondrial quantity and density have been linked with increased endurance performance (Psilander et al., 2013) and a reduction in type 2 diabetes (Lee et al., 1998, De Feyter et al., 2008, Patti et al., 2003, Petersen et al., 2004, Mootha et al.,

2003). Also, it is suggested that dietary strategies that maintain LM are preferential. This is the first study to demonstrate the impact of increased protein intake in a hypocaloric diet on metabolic adaptation. The results from this study demonstrated that a 7-day a high protein hypocaloric diet resulted in increased AMPK, SIRT1, SIRT3 and PGC-1α mRNA expression at rest, all of which are upstream markers of mitochondrial biogenesis, however no between group effect was observed. Additionally when maintaining macronutrient ratio and reducing energy intake, a high protein diet attenuated LM loss greater than a high carbohydrate diet. Visual trends suggest that a high protein, hypocaloric diet is preferential to a high carbohydrate, hypocaloric diet with respect to cellular markers of fat metabolism and mitochondrial biogenesis, also the maintenance of lean mass, however post-hoc power calculations suggest the study was underpowered to identify the true difference. Further larger studies are required to investigate this further, additionally future research is needed to understand the long-term impact of a high protein hypocaloric diet on markers of mitochondrial biogenesis and body composition.

6.6 Limitations.

- The study was over a fixed acute period of time of 7-days and demonstrated a metabolic effect. Measurements were collected pre and post; as such the time course of the observed changes cannot be defined from these results. More research is required to define the speed and amplitude of the changes observed.
- Participants were not observed throughout the trial. Every effort was made to provide diets matched to the intervention requirements, but the participants were not monitored daily throughout the trial. Thus assumptions have to be made that dietary adherence was followed.
- It has previously been demonstrated that increased mRNA expression (measured in this study) is associated with increased acetylation and phosphorylation of the genes, however due to the muscle sampling technique this was not able to be measured.
- The transcriptional mRNA measured in this study are upstream markers of adaptation, they demonstrate the potential to adapt. Adaptation per se was not directly assessed.

6.7 Future research and next experimental Chapter

The results have demonstrated that increasing metabolic stress by calorie and carbohydrate restriction positively promotes AMPK, SIRT1 and PGC-1α mRNA expression, all of which are metabolic markers of mitochondrial biogenesis. It has previously been demonstrated that endurance exercise is also a potent stimulator of such genes. However *in vivo* the combination of a high protein diet and endurance training is yet to be fully quantified, investigating this help provide further understanding of the mechanisms and stimulators of mitochondrial biogenesis *in vivo*.

7 THE IMPACT OF A 7-DAY HIGH PROTEIN DIET ON CELLULAR ADAPTATION, SUBSTRATE UTILISATION AND ENDURANCE RUNNING PERFORMANCE IN WELL-TRAINED RUNNERS.

Background: It has been demonstrated that transcriptional markers of mitochondrial biogenesis are sensitive to calorie restriction and a high protein low carbohydrate diet (HP/LC). The aim of this experimental Chapter was to explore the impact of a HP/LC diet in combination with endurance training on skeletal muscle metabolic adaptation, substrate utilisation and exercise performance. **Method:** In a parallel groups repeated measures study 16 well trained endurance runners ($\hat{V}O_2max$ = 64.2 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$) were randomised into two groups: High protein low carbohydrate (PRO) or a high carbohydrate low protein (CHO). Participants completed 3 performance trials each separated by 7 days, visit 1 after habitual diet, visit 2 after intervention diet and visit 3 after habitual diet. Each visit a resting muscle biopsy was collected followed by a 10 km steady state (SS) run and a time to exhaustion (TTE) trial at 95% $v\dot{V}O_2max$. **Results:** A significant increase in AMPK (1.44 and 1.57 fold increase) and PPAR (1.32 and 1.29 fold increase) mRNA expression was observed post intervention and remained elevated in the CHO group. AMPK (1.37 fold increase) was elevated post intervention in the PRO group which returned to baseline levels subsequently. Following the intervention diet the PRO group exhibited a 101 % increase in fat oxidation and 27 % decrease in carbohydrate oxidation during the 10 km SS run ($p < 0.001$), no change was observed in oxidation rates in the CHO group. A significant decrease in TTE was observed subsequent to the PRO intervention (-23.3%, $p = 0.0003$), no change was observed in the CHO group (6.5%, $p = 0.051$). **Conclusion:** A 7-day high protein diet significantly increases sub-maximal fat oxidation, this change was reverted back to pre-levels once returned to habitual diet for 7 day. The CHO group promoted the greatest positive changes in mRNA transcriptional markers, however only small changes were observed. The PRO diet significantly compromised the ability to complete high intensity running.

7.1 Introduction

Athletes cannot train indefinitely. Implementing an intervention which increases the metabolic stress of a training session, potentially leading to enhanced adaption and improving subsequent exercise performance can be attractive to an athlete (Holloszy and Coyle, 1984). It is established that endogenous carbohydrate stores are only sufficient to fuel ~3 hours of sub-maximal exercise performance (Burke and Hawley, 2002), whereas fat stores can provide enough fuel for multiple days of low intensity activity (Rapoport, 2010). Numerous studies have demonstrated that improving exercise efficiency and sparing muscle glycogen can enhance endurance performance (Coyle et al., 1986), additionally it has been shown that well-trained athletes have a greater ability to oxidise fat during prolonged moderate-intensity exercise (1986). There is evidence showing that a high fat, low carbohydrate diet, in combination with endurance training, enhances sub-maximal fat oxidation (Miller et al., 1984, Lambert et al., 1994, Goedecke et al., 1999, Burke, 2015). However the optimal dietary manipulation strategy to enhance metabolic adaptation has not yet been defined, and more importantly for athletes, the impact this has on high intensity performance is inconclusive (Hawley and Morton, 2014).

With respect to altitude training it is established that the benefits attained through training in hypoxic conditions are exhausted within 14–21 days return to sea level (Chapman et al., 2014). Within the current area of research, the primary aims of the studies were focused on performance immediately post an acute low carbohydrate diet. The longer term impact of continuous restricted carbohydrate intake on substrate utilisation, metabolic adaptation and performance are yet to be fully quantified, and importantly the effect of returning back to habitual dietary on such markers is not fully understood.

Mitochondrial density and abundance has been linked to improved aerobic capacity, energy metabolism and endurance performance all of which are desired adaptations from endurance training (Hawley and Morton, 2014). This is discussed in detail in Chapter 2.2.2 but briefly, it is fairly well established that a series of transcriptional genes play an essential role in the activation of mitochondrial biogenesis and fatty acid oxidation, including AMPK, SIRT1, PPAR and the master regulator PGC-1α

(Ruderman et al., 2010). A substantial body of evidence has demonstrated that SIRT1 and AMPK both respond to increases in energy expenditure and reduced carbohydrate availability and it has been suggested that increased dietary carbohydrate intake prevents the activation of the AMPK-SIRT1 axis in skeletal muscle (Draznin et al., 2012). As discussed earlier (Chapter 2.3.1) it was previously accepted that high fat, low carbohydrate diets induce favorable shifts in substrate utilisation, promoting greater fat oxidation, but subsequent high intensity exercise performance is not enhanced and may be compromised (Burke and Hawley, 2002). However the discovery of PGC-1α and the mechanistic understanding developed over the last 10 years has provided a platform to develop new strategies which lead to increased transcriptional markers of mitochondrial biogenesis, fatty acid oxidation and potentially exercise performance (Hawley and Morton, 2014).

The results in the previous experimental Chapter of this thesis demonstrated that a high protein, hypocaloric diet significantly increased the expression of AMPK, PPAR and PGC-1α at rest. This response was blunted in the high carbohydrate and eucaloric groups suggesting that carbohydrate and energy availability blunt the metabolic response. Similar results were observed by Draznin et al. (2012) when feeding a high fat diet. Increased protein feedings have also been demonstrated to increase the fat to lean mass ratio during weight loss (Layman et al., 2003), a favorable adaptation for endurance runners. Carbohydrate restriction is generally achieved from increased dietary fat intake and it is common for these diets to be poorly tolerated (Bartlett et al., 2015), the impact of increased protein as a method to restrict carbohydrate intake during endurance performance is yet to be fully explored. As such the aims of this experimental Chapter were to investigate the impact of a high protein diet, in combination with endurance training, on cellular, metabolic and performance markers during sub-maximal and maximal running performance, and also to investigate the impact of returning to a habitual diet on any observed adaptations/changes.

7.1.1 Aims and Objectives

There are two primary aims from this phase of research:

- To investigate the impact of a 7-day high protein or high carbohydrate diet, in combination with endurance training, on skeletal muscle adaptation, substrate utilisation and running exercise performance.
- To observe the response of returning back to habitual dietary intake following a 7-day high protein or high carbohydrate diet on skeletal muscle adaptation, substrate utilisation and exercise performance

The following hypotheses have been made for this body of research:

i) (**H_1**) A 7-day high protein, low carbohydrate diet, in combination with endurance training, will promote positive cellular transcriptional adaptation to a greater extent than a high carbohydrate, low protein diet.
(**H_0**) No between group differences in cellular transcriptional response will be apparent.
ii) (**H_2**) A 7-day high protein, low carbohydrate diet, in combination with endurance training will significantly increase sub-maximal fat utilisation.
(**H_0**) No time point or between group differences in sub maximal substrate utilisation will be apparent.
iii) (**H_3**) 7-days consuming a high protein low, carbohydrate diet will result in a decrease in high-intensity exercise performance.
(**H_0**) No time point differences in maximal exercise performance will be apparent.
iv) (**H_4**) Adaptations occurring subsequent to consuming a high protein will be maintained once reverted back to habitual dietary intake.
(**H_0**) No observed adaptations will remain subsequent to returning to habitual diet for 7-days.

7.2 Method

7.2.1 Participants

Twenty healthy males volunteered to participate in the study, with sixteen completing (mean ± SD: age; 26.3 ± 4.5 years, body mass; 68.6 ± 4.5 kg, height; 180.4 ± 5.2 cm, $\dot{V}O_2max$; 64.2 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$). Two participants dropped out due to injury, one dropped out as a result of illness and one was unable to start the second trial following 7-days on the high protein diet. Participants were screened via telephone against pre-determined criteria to ensure the inclusion criteria were met and were free from any medical condition that would preclude their participant in the study. Participants could

not be following a restrictive dietary regime or suffer from any food allergies or intolerances. Participants must have run 10 km under 38 minutes or 5 km under 18 minutes in the past 12 months, with a running history > 2 years, training ≥ 4 times per week with no significant change in training load or body mass in the previous 3 months. The experimental procedures and potential risks associated with the study were explained. Written informed consent was attained prior to the start of the study. The study was approved by the University of Hertfordshire Life and Medical Sciences Ethics Committee, protocol number LMS/PGR/UH/02227.

7.2.2 Study Protocol

In a randomised, experimenter-blinded, parallel groups, repeated-measures study design, participants were assigned to one of two groups (Table 7.2.1); either energy matched high protein (PRO) or energy matched high carbohydrate (CHO) (Table 7.2.2). The participants were not informed of group allocation, however due to the nature of dietary intervention studies it was not possible to blind food items. The participants attended the laboratory on four separate occasions, each separated by 7-days (Figure 7.2.1). Visit 1 was for familiarisation. Between visits 1 and 2 participants remained on their habitual diet and stable training programme, between visits 2 and visit 3 participants consumed the prescribed intervention diet and between visits 3 and 4 participants returned to their habitual diet. The participants were requested to complete the same training sessions each week throughout the duration of the study and rest the day prior to each visit. All experimental procedures were completed in the same order at the same time of the day.

Table 7.2.1 Group characteristics.

Group	Height (cm)	Weight (kg)	Absolute $\dot{V}O_2max$ ($L \cdot min^{-1}$)
PRO	179.4 ± 6.4	69.5 ± 3.3	4.38 ± 0.35
CHO	181.6 ± 3.5	67.6 ± 6.1	4.39 ± 0.28

Table 7.2.2. Prescribed macronutrient ratio for the intervention diets.

	Carbohydrate (%)	Protein (%)	Fat (%)
PRO (n= 8)	30	40	30
CHO (n = 8)	60	10	30

PRO = Energy matched high protein diet, CHO = Energy matched high carbohydrate diet.

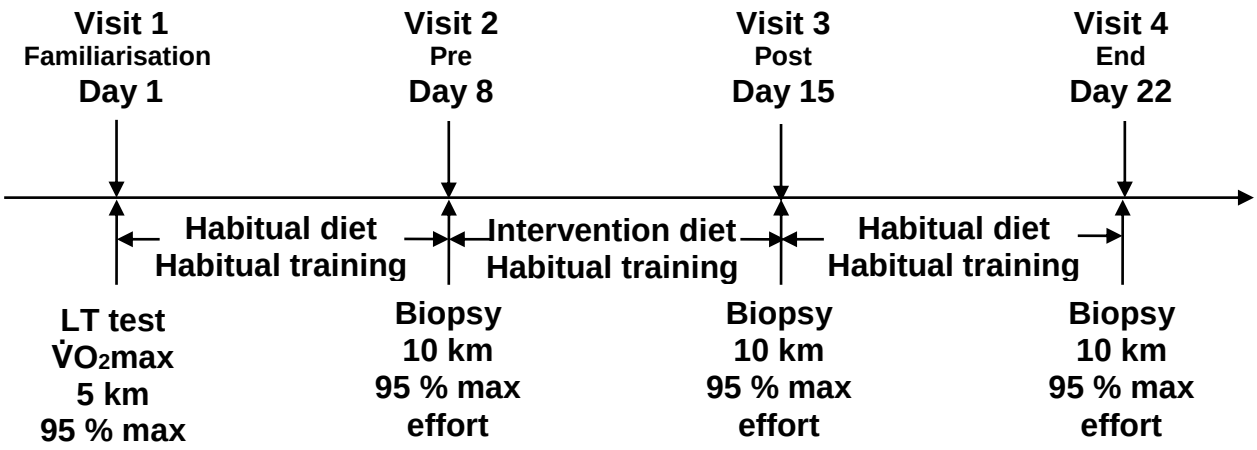


Figure 7.2.1. Schematic of study design.

Inclusion criteria: Male aged between 18 – 35 years and has a 10 km running time ≤ 38 minutes or 5 km running time ≤ 18 minutes which has been achieved in the last 48 months, is currently training ≥ 4 times per week and has no major training disruption in the preceding 3 months. Participants must be in good general health with no identified chronic illness and currently free from cold, flu or any musculoskeletal injury.

Exclusion criteria: If any medication has been taken within 14 days of the study start or any regular use of over the counter herbal medication (occasional dosages of paracetamol for pain relief is permitted). If currently following a restrictive dietary regime which excludes any food groups (e.g. Vegan, Vegetarian), or if the participant is aware of any food allergies. If consuming any nutrition supplements (or unwilling to

abstain from taking during the study), or on medication (beta-blockers, NSAID's or recreational drugs).

7.2.3 Experimental protocol.

Following a 4-hour (minimum) fast, participants arrived at the laboratory at the same time of day for each visit. Participants were advised to arrive at the laboratory using the same mode of transport and requested to refrain from morning physical exertion. Upon arrival a body composition (DXA scan) assessment was undertaken, followed by a micro muscle-biopsy. Participants then completed a 10km run at 75% $\dot{V}O_2$max, had five minutes rest, then completed a time to exhaustion (TTE) running trial at 95% v$\dot{V}O_2$max. All exercise testing was performed in the same temperature controlled room (20°C; 35% humidity), on the same treadmill, using the same online gas analyser (refer to Chapter 3.7 and 3.8 for the calibration and reliability data of the online gas analyser and the treadmill).

7.2.4 Familiarisation:

Following the study briefing and paperwork completion, participants were shown the DXA scanner and the scanning protocol was described to them. Participants then changed into running clothing. A resting whole blood capillary sample was collected from the finger and height and weight were recorded. The participant then completed a standardised 5-minute warm up at a running speed of 9 km•h^{-1}. If desired the participant completed self-directed stretching. Participants were fitted with a HR belt (Polar T31 Coded, Polar Electro Ltd, Warwick, UK) and face mask and the corresponding participant details were entered in to MetaSoft® Studio software (Cortex, Leipzig, Germany). Via the face mask, respiratory data was collected using a breath by breath online gas analyser (Metalyzer 3B (Cortex, Leipzig, Germany). All testing was completed on one treadmill (Cosmed T170, Cosmed, Rome, Italy), which was not moved throughout the experimental period. Fan cooling was provided throughout all testing; a floor fan was positioned on the floor in front of the treadmill at a 45° angle and set to an air speed of ~5.8 m•s^{-1}. The same lactate threshold protocol

was used for all participants: the treadmill was set to a 1% gradient and belt speed set at 11 km•h^{-1}, participants straddled the belt and once up to speed started to run on the treadmill. Stage increments of 1 km•h^{-1} were used and each stage lasted 4 minutes. Expired gas and HR were collected throughout, rating of perceived exertion (RPE) (Borg, 1970) was collected at 3 minutes into each stage, at 3 minutes 30 seconds participants were requested to straddle the belt and a finger prick capillary blood sample was taken. After collection participants started running again and the next stage commenced. The capillary blood sample was analysed immediately using an EKF Biosen C line (EFK, Barleben, Germany) (refer to Chapter 3.9 for details), cessation of the LT test occurred when blood lactate had reached > 4 mmol•L^{-1} and or > 3 mmol•L^{-1} above resting blood lactate. A standardised 10 minutes rest between the LT and $\dot{V}O_2$max test followed.

The protocol for the $\dot{V}O_2$max test was the same for all participants. The treadmill was set to a 1% gradient, the participant was re-fitted with the HR belt and face mask. The test started at 12 km•h^{-1} and increased at a gradual velocity of 1 km•h^{-1} every minute, the test protocol was programmed in to MetaSoft® Studio and controlled automatically. Expired gas and HR was collected throughout. Cessation of the test occurred upon volitional fatigue. Verbal encouragement was allowed throughout. Using the MetaSoft® Studio software the data output was selected to provide a 60 second rolling average of the $\dot{V}O_2$ (L•min^{-1}) values. The highest 60 second rolling average was selected and calculated to a relative $\dot{V}O_2$max (mL•kg^{-1}•min^{-1}) for the participant:

$$\dot{V}O_2\text{max (mL•kg}^{-1}\text{•min}^{-1}) = (\dot{V}O_2\text{ (L•min}^{-1}\text{) x 1000) / Body mass (kg)}$$

Participants had 20 minutes recovery, during this period ad-lib water consumption was permitted. Following this rest period a 5 km steady state (SS) familiarisation run at 70% v$\dot{V}O_2$max (~75% $\dot{V}O_2$max) was completed under the same conditions as the 10 km SS exercise trial detailed below (Chapter 7.2.8.3). Once completed, participants had 5 minutes recovery then completed a maximal sustainable effort at 95 % v$\dot{V}O_2$max following the protocol detailed below (Chapter 7.2.8.4). Prior to departure participants were provided with a food dairy (refer to Chapter 3.1) and a GPS watch (Garmin Vivo

Active, Garmin Ltd, Schaffhausen, Switzerland) to log dietary intake and training volume.

7.2.5 Running Training

It was important that any observed changes were due to the intervention diet and not a deliberate change in training volume; as such training volume was controlled each training week. Participants were requested to keep to the same weekly training programme throughout the 3-week study and were requested to replicate, as closely as possible, each training session on the same day and time each week, logging all training sessions with the GPS watch provided. The training sessions were automatically uploaded online (Garmin Connect, Garmin Ltd, Schaffhausen, Switzerland) for the investigative team to monitor and download for later analysis.

7.2.6 Habitual Dietary Intake.

A 4-day food record using estimated household measures was used to collect habitual dietary intake prior to the first and third testing sessions. Refer to Chapter 3.1 for details describing the diet log process. Participants were requested to record dietary intake on three week days and one weekend day between visit 1 and visit 2, repeating this again between visit 3 and visit 4.

7.2.7 Intervention Dietary prescription

Individual calorie intake for the intervention diet was calculated using basal metabolic rate (BMR) multiplied by a physical activity factor (PAF). The Henry (2005) equation used to calculate BMR:

“BMR = 14.4 x weight (kg) + 313 x height (m) + 113”

PAF was calculated depended on occupational and exercise level using a modified Harris and Benedict (1918) (Nutritics) equation:

"PAL = (0.66 x occupational factor) + (1.33 x exercise factor)

Table 7.2.3. Occupational and exercise physical activity multiplication factors.

Occupation level	Multiplication factor
Sedentary	1.2
Lightly	1.375
Moderately	1.55
Very	1.725
Extremely	1.9

Exercise level	Multiplication factor
Light	1.375
Moderate	1.55
Very	1.725
Extra	1.9

Unless training twice a day the participant was deemed to be 'Very' active, if training twice a day 'Extra' active was selected. Occupational multiplication factor was selected through a series of work related questions. The calculated required energy intake was then used to prescribe dietary intake as described in Chapter 3.3. As demonstrated in the results in experimental Chapter 6, calculating and matching energy intake to energy expenditure is challenging. It was important that the participants were not in a negative energy balance for this study, therefore in addition to the calculated diet intake, a further 500 kcal•day^{-1} macronutrient-matched meal was provided. Participants were briefed that this was only to be consumed if hungry after consuming the standard diet and were required to document if this additional meal was consumed. Participants were required to refrain from consuming any additional food and requested to drink water or drinks free from caffeine or additional energy.

Table 7.2.4. Calculated mean dietary intake and macro nutrient breakdown for each group during the 7-day intervention.

Group	Dietary intake (kcal•day^{-1})	CHO (•day^{-1})		PRO (•day^{-1})		FAT (•day^{-1})	
		g	g•kg^{-1}	g	g•kg^{-1}	g	g•kg^{-1}
PRO	3185 ± 237	239 ± 18	3.4 ± 0.3	319 ± 24	4.6 ± 0.3	106 ± 8	1.5 ± 0.1
CHO	3281 ± 195	492 ± 29	7.3 ± 0.4	82 ± 5	1.2 ± 0.1	109 ± 7	1.5 ± 0.1

Prescribed dietary intake (kcal•day^{-1}, mean ± SD) and macronutrient breakdown for the prescribed intervention diets for both groups.

7.2.8 Testing visit:

7.2.8.1 Body composition assessment: Dual energy x-ray absorptiometry (DXA) scan.

Body composition was measured using a DXA scanner; refer to Chapter 3.6 for descriptions of the procedure.

7.2.8.2 Micro Muscle-Biopsy

Muscle tissue was attained using the micro muscle biopsy technique; refer to Chapter 3.4 for details describing the procedure.

7.2.8.3 10 km steady state treadmill run

Following a resting capillary blood sample, participants completed a standardised warm-up consisting of a 5 minutes jog at 10 km•h^{-1} on the treadmill, followed by self-directed stretching. A facemask (attached to the online gas analyser) was fitted and stayed on throughout the trial. Participants were asked to straddle the treadmill and the belt speed was set to the intensity representative of 75% $\dot{V}O_2$max (as calculated from the familiarisation session). Once the treadmill belt was up to speed participants, whilst holding on to the treadmill arms, stepped onto the belt and began running. As soon as the participants let go of the treadmill arms the time was noted and the sub-maximal exercise trial started. Participants had minimal visual or audible stimulus throughout the trial. After each kilometer was completed the same words were repeated to the participant: “Well done that is another kilometer completed, ‘X’ to go”, a capillary blood sample and RPE were then collected. Participants were also informed of the distance completed at 9,500 meters. At 9,850 meters the final capillary blood sample and RPE were collected. As soon as the 10 kilometer run was completed

participants held onto the arms of the treadmill, and straddled the belt. A stopwatch was started immediately to time 5-minute recovery. The facemask was removed and participants stepped off the treadmill (participants were permitted to walk and go to the toilet if required during the recovery).

7.2.8.4 95% maximal sustainable effort (MaxSE).

As a means to justify the use of MaxSE as a measure of endurance performance. In the London 2012 men's 800 meter Olympic final 1.16 seconds separated the 8 runners after 400 meters, with the leader going through in 49.28 seconds, a second lap of 51.63 seconds by the winner resulted in a world record time. The remaining field who all ran, or were close to personal bests were separated by 2.86 seconds; the athletes that slowed the least won. As with this example most middle distance running events can be described as a maximal sustainable effort, as such the maximal sustainable effort is a fair assessment of high intensity running performance.

The treadmill belt was set to 95% v$\dot{V}O_2$max (as calculated from the familiarisation session), participants straddled the belt and the face mask was fitted again. Once the 5 minutes recovery was up participants supported their weight on the arms of the treadmill and started running on the belt. Once up to speed participants let go and the stopwatch was started to record the duration of the effort. No verbal or audible encouragement was permitted throughout the trial. Participants ran until volitional fatigue, once reached participants straddled the treadmill. The stopwatch was stopped as soon as the participant reached out for the treadmill arms to support their weight. Immediately upon volitional fatigue a final capillary blood sample was collected. The same investigator timed all MaxSE efforts throughout the study. The coefficient of variation (CV) of the MaxSE trial used in this study from 8 participants over two trials, separated by 14-days, was 5.6%.

7.2.9 Analysis

7.2.10 Calculation of contribution of energy expenditure

The aerobic and anaerobic contributions towards the energy expenditure of the exercise trial were calculated using the following equations from Hill (1999):

"Energy cost of running = (Aerobic O_2 + 2.3) + (PCr contribution) + (glycolytic contribution)"

- Aerobic O_2 = Total O_2 consumption mL•kg^{-1}
- PCr contribution = 37 mL O_2•kg^{-1} muscle mass^{-1}
- Glycolytic contribution = 3 mL•kg body mass^{-1} per mmol•L^{-1} increase in lactate above resting.

Muscle mass was calculated using the equations from Kim et al. (2002), the method states an *r* value of 0.97 compared to magnetic resonance imaging (MRI) and report a mean standard error of estimate of 1.63 kg.

"Total body skeletal muscle mass (SMM) (kg) = (1.13 x ALST) – 0.02 x age) + (0.61 x sex) + 0.97"

ALST = Total lean tissue of both right and left arms and legs (As quantified by DXA scan, describe in Chapter 3.6). Sex: Female = 0, Male = 1

For example, the measured values for the MaxSE trial on visit 1 for participant 008:
Weight (kg) = 73.3
SMM (kg) = 35.2
Total oxygen consumption (mL•kg^{-1}) = 134.4
Resting lactate (mmol•L^{-1}) = 1.2
Max lactate mmol•L^{-1}) = 8.4

Therefore the total cost of running and subsequent aerobic and anaerobic components are:

Energy cost of running = (134.4 + 2.3) + (37 x 35.2/73.3) + ((8.4 – 1.2) x 3) = 176.2 mL O_2•kg^{-1} (77.6% aerobic and 22.4% anaerobic).

7.2.11 Quantitative PCR (qPCR) preparation and analysis.

Refer to Chapter 3.5 for the description of RNA isolation, reverse transcription, qPCR analysis and the application efficiency techniques used in this study.

7.2.12 Data analysis of the qPCR assays

The muscle biopsy qPCR raw Cp values were exported and normalised to the housekeeper gene (GAPDH) using an analysis of covariance method. Relative fold change in expression levels over testing visit one were then calculated using $2^{-\Delta\Delta C_T}$ method.

7.2.13 Statistical analysis.

Kolmogorov-Smirnov tests of normality to assess the null hypothesis that data for within-subjects analysis was normally distributed. However it should be noted that the ANOVA procedure is robust to slight violations of normality within a dataset (Field, 2013). Mauchly's sphericity test was used to assess the assumption of sphericity within repeated-measures effects. Unless stated otherwise Mauchly's test was insignificant ($p > 0.05$), therefore the assumption of sphericity was accepted.

Group and time point effects were assessed using a two factorial mixed design ANOVA (4 groups, 2 time points) with dietary intake (between) and time point (within) as the main variables. To determine the location of the time point differences and group interactions a Bonferroni post hoc test was used. Statistical significance was set

at $p \leq 0.05$. Statistical analysis was conducted using the statistical package for the Social Science software program (SPSS; version 22, IBM, Armonk, NY). Data presented as mean ± SEMs (unless stated).

7.3 Results

No group difference was observed in total training distance each week ($p = 0.83$), nor were there any significant difference across any time points. However a trend towards a reduction in average running speed was observed between week 1 and week 2 in the PRO group (Table 7.3.1). In week 1 the mean speed of the training runs was 14.7 km•h^{-1} which decreased to 13.8 km•h^{-1} following the high protein intervention diet, and increased back to 14.2 km•h^{-1} once reverted back to habitual diet.

Table 7.3.1. Running distance and speed during each week of training throughout the duration of the study.

	Week 1		**Week 2**		**Week 3**	
	Distance (km)	Average pace (km•h^{-1})	Distance (km)	Average pace (km•h^{-1})	Distance (km)	Average pace (km•h^{-1})
PRO	51 ± 26	14.7 ± 1.7	51 ± 28	13.8 ± 1.5	50 ± 28	14.2 ± 1.3
CHO	62 ± 20	13.9 ± 0.8	64 ± 24	14.1 ± 1.4	65 ± 22	13.9 ± 0.9

No change was observed in the resting mRNA content of PGC-1α, SIRT1 and SIRT3 at either time point in either group (Figure 7.3.1 A, C and D). However a 7-day high carbohydrate diet resulted in a significant increase in resting AMPK mRNA expression (1.44 fold increase relative to TP1, $p < 0.05$), the elevated mRNA expression was maintained at TP3 (1.57 fold increase relative to TP1, $p < 0.05$) (Figure 7.3.1 B). The PRO group exhibited a similar significant increase in AMPK mRNA expression following the intervention diet (1.37 fold increase relative to TP1, $p < 0.05$); however by TP3 it had returned back to TP1 levels. Within the CHO group the same trend observed in AMPK was apparent for PPAR resting mRNA expression, with a 1.32 and 1.29 fold increase at TP2 and TP3 ($p < 0.05$) relative to TP1 respectively. No time point interaction in PPAR was observed in the PRO group (Figure 7.3.1 E).

Table 7.3.2. Habitual dietary intake during the study weeks with no dietary intervention (mean ± SD and percentage (%) of total energy intake).

	Group	Daily calorie intake (kcal•day^{-1})	Carbohydrate		Protein		Fat	
			grams	%	grams	%	grams	%
Week 1	**PRO**	2382 ± 895	292	50	119	20	78	30
	CHO	2683 ± 471	352	49	129	19	102	32
Week 3	**PRO**	2419 ± 1041	300	49	120	20	90	31
	CHO	2648 ± 461	319	48	116	18	105	34

The relative running intensity during the 10 km SS run was 75.6 ± 2.6 % $\dot{V}O_2$max, no difference between groups was observed (PRO = 76.4 ± 3.2% and CHO = 74.7 ± 2.4% $\dot{V}O_2$max). No difference in carbohydrate and fat oxidation was observed at any time point during the 10 km SS trial across all three trials in the CHO group (Figure 7.3.2 B and D). Whereas at time point 2 (following the intervention diet) a significant change ($p < 0.0001$) in substrate utilisation was exhibited in the PRO group (Figure 7.3.2 A and C). Mean fat oxidation increased from 0.29 ± 0.05 to 0.59 ± 0.05 g•min^{-1}, an increase of 101% relative to trial 1 (Figure 7.3.2 A). Additionally at time point 2, a significant ($p < 0.001$) decrease of 27% (2.96 ± 0.18 to 2.15 ± 0.19 g•min^{-1}) was observed in carbohydrate oxidation. Both fat and carbohydrate oxidation returned to pre intervention levels once reverted back to habitual dietary intake (Figure 7.3.2 A and C).

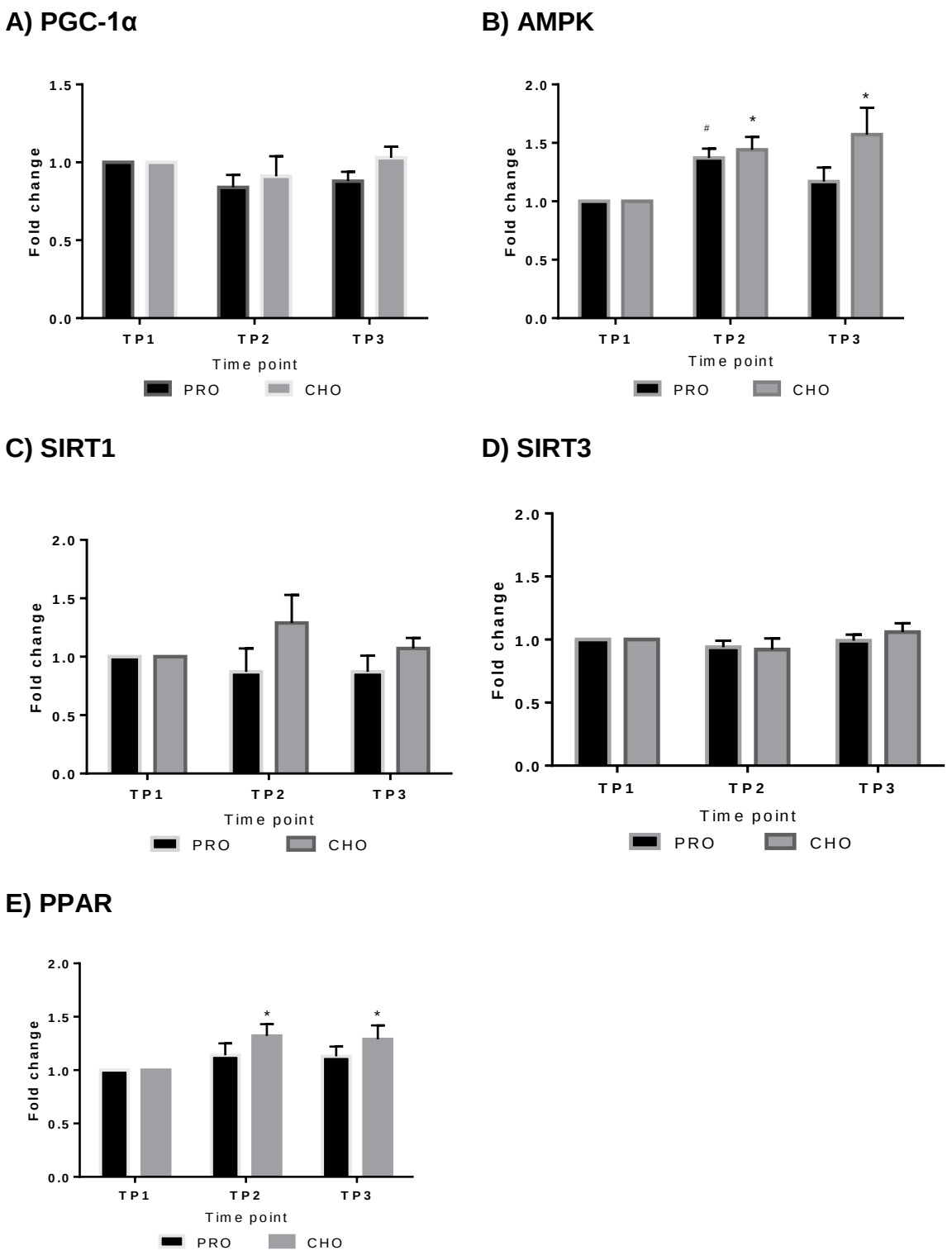


Figure 7.3.1.mRNA response (fold change from pre) to 7 day dietary intervention in combination with endurance training.

*TP1 and TP3 are subsequent to 7 days habitual diet, TP2 is subsequent to either a high carbohydrate (CHO) or high protein (PRO) intervention diet. mRNA expression of PGC-1α (**A**), AMPK (**B**), SIRT1 (**C**), SIRT3 (**D**) and PPAR (**E**).* Denotes significantly different from TP1 ($p < 0.05$), (mean ± SEM).*

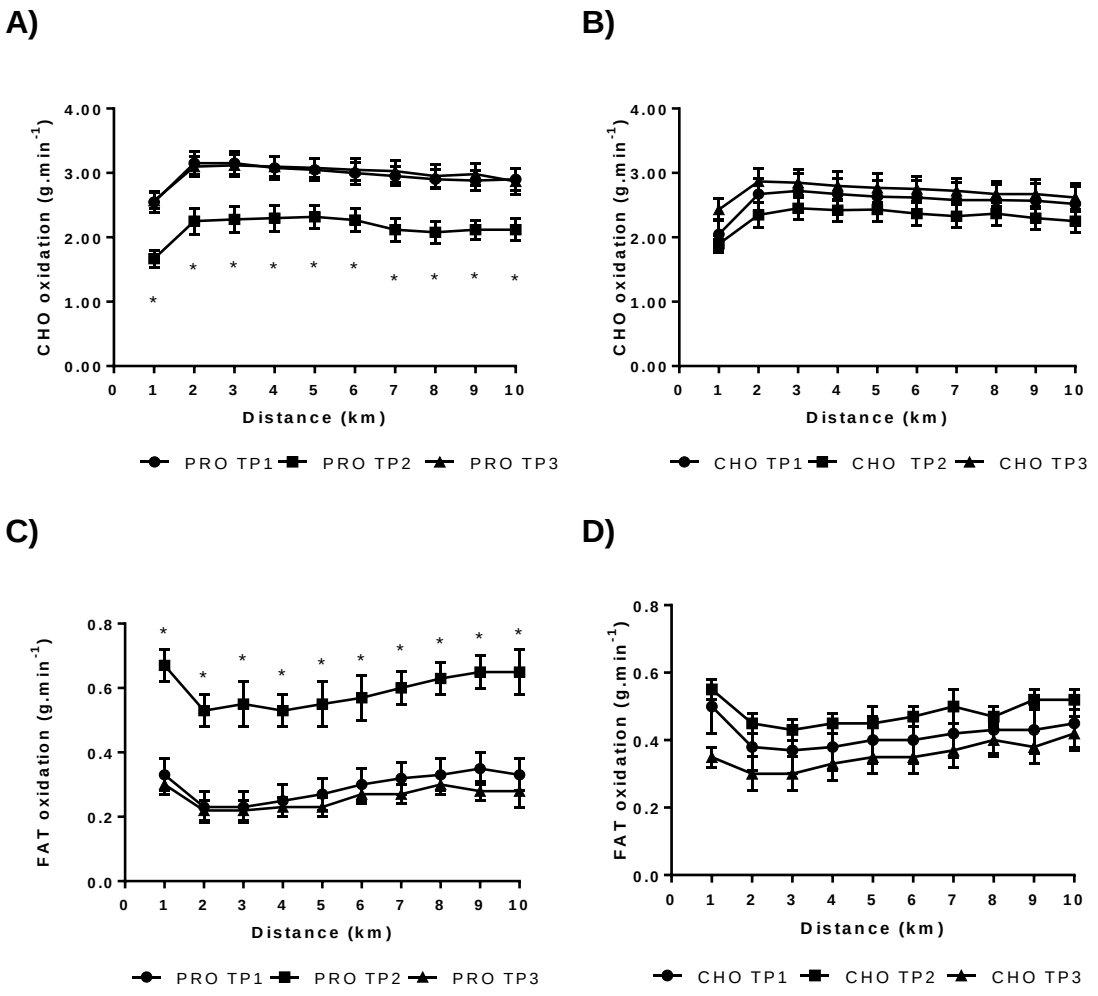


Figure 7.3.2. Substrate oxidation rates during a 10 km steady state (~75% $\dot{V}O_2max$) treadmill run at three time points.

*The data demonstrated at TP1 and TP3 are subsequent to 7 days habitual diet, the data for TP2 is subsequent to 7-days intervention (high carbohydrate (CHO) or high protein (PRO)). Carbohydrate oxidation (g•min^{-1}) in the high protein group (**A**), carbohydrate oxidation in the high carbohydrate group (**B**), fat oxidation in the high protein group (**C**) and fat oxidation in the high carbohydrate group (**D**). * Denotes significant difference compared to both TP1 and TP3 ($p < 0.05$) (mean ± SEM).*

The results of the 95% MaxSE trial demonstrated a non-significant (p = 0.051) increase of 6.5% in TTE at TP2 in the CHO group with 7 of the 8 participants improving TTE relative to TP1 (Figure 7.3.3 B), this was returned to pre intervention levels at TP3 (182.2 ± 44.4 vs. 184 ± 45.5 vs. 184 ± 38.9 seconds). On the contrary, TTE was significantly reduced in the PRO group and at TP2 (-23.3%, p = 0.0003) following the dietary intervention and all 8 participants exhibited a decrease in TTE (Figure 7.3.3 A), upon returning to habitual diet TTE returned to TP1 performance (128.3 ± 29.3 vs. 98.4 ± 31.8 vs.125.3 ± 32.39 seconds) (Figure 7.3.3 A).

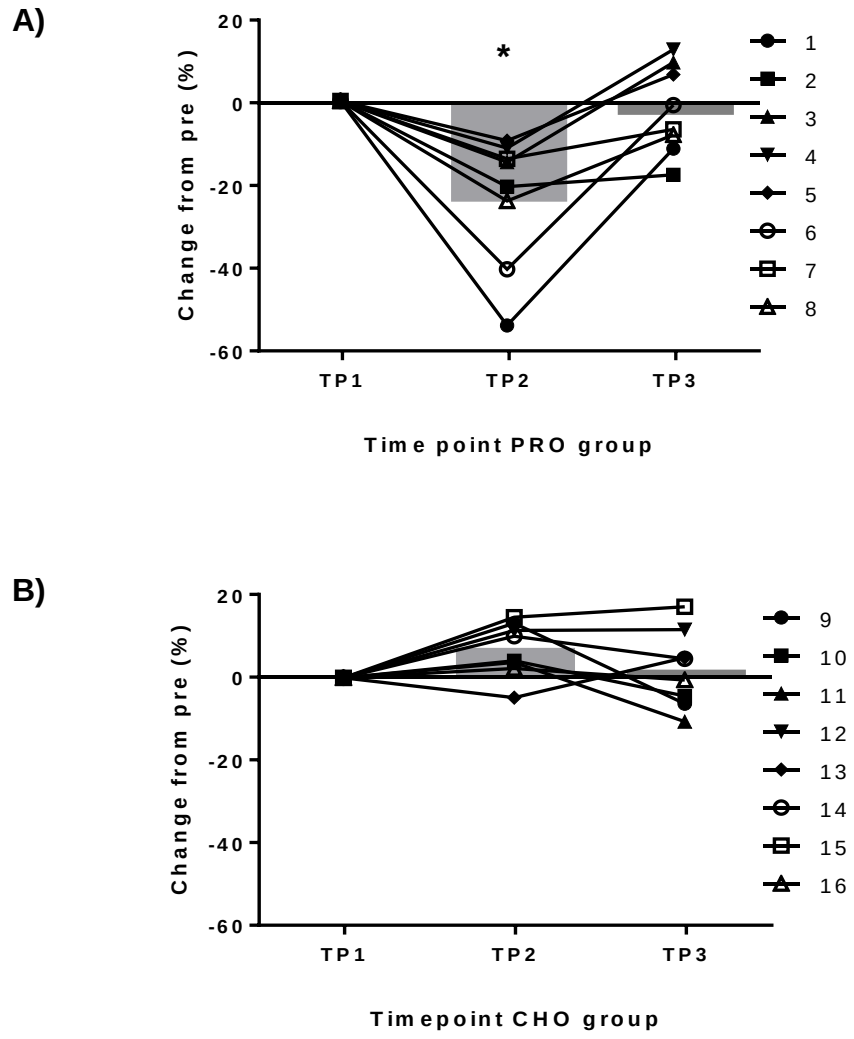


Figure 7.3.3. Mean (bar) and individual (line) relative change (%) from pre intervention maximal sustainable effort.

*TTE from a 95% MaxSE trial. TP1 and TP3 are subsequent to 7 days habitual diet, TP2 is subsequent to either a high protein (**A**) or high carbohydrate (**B**) intervention diet.* = significant difference (p < 0.05) compared to TP1.*

Dietary intake was calculated to match energy expenditure. No significant time point differences were observed in body mass, lean mass or fat mass in either group (Figure 7.3.4 A, B and C), demonstrating energy intake matched energy expenditure during both the dietary intervention and habitual intake weeks.

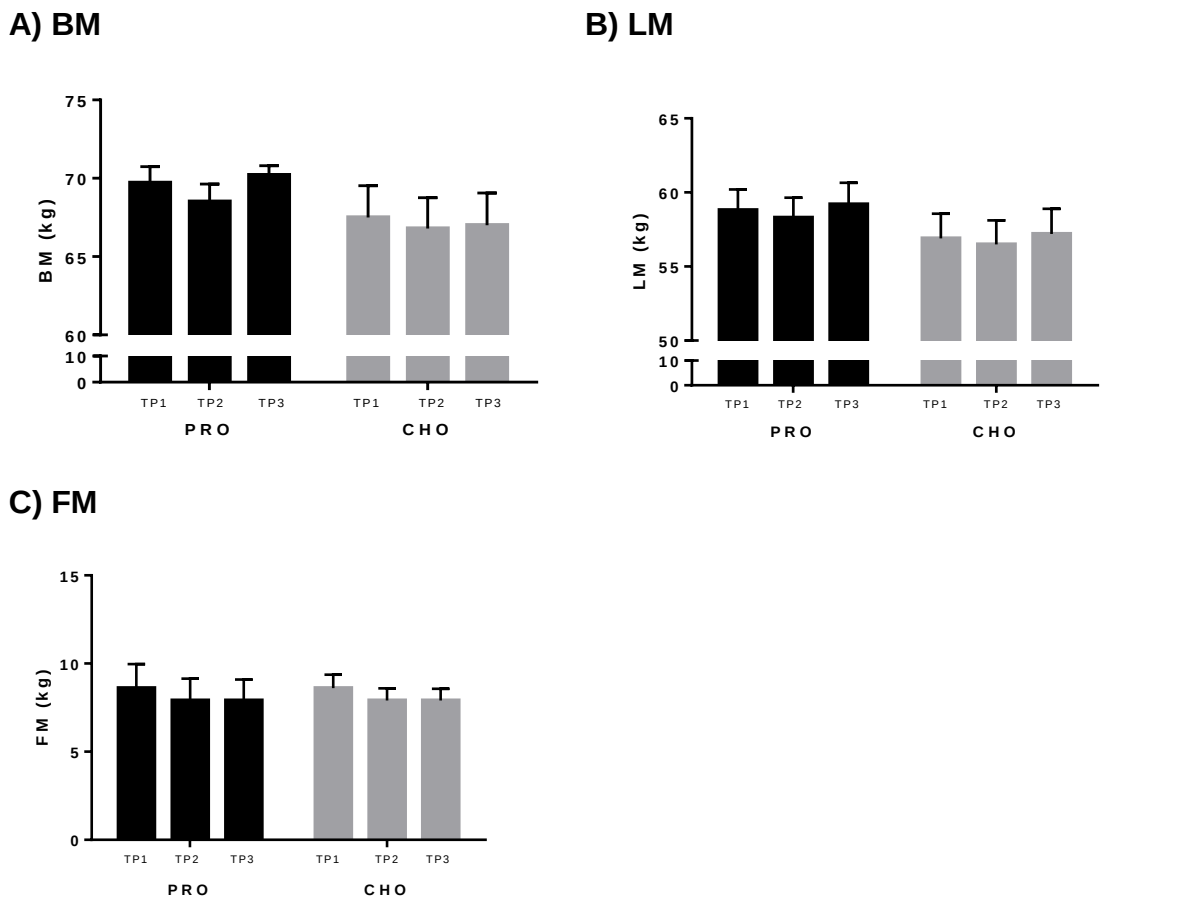


Figure 7.3.4. Body composition changes subsequent to dietary intervention.

*Body composition; body mass (BM) (**A**), lean mass (LM) (**B**) and lat mass (FM) (**C**) following either 7-days habitual diet (TP1 and TP3) and 7-days intervention diet (high carbohydrate (CHO) or a high protein (PRO)) (TP2).*

With regards to RPE, blood lactate and blood glucose, no time point difference was observed during 10 km SS trial in the CHO group (Figure 7.3.5 A – F). Resting blood glucose was significantly ($p < 0.05$) lower in the PRO group following the intervention diet compared to both time point 1 and 3, this was the only difference observed (4.1 ± 0.4 vs. 4.7 ± 0.6 and 4.9 ± 0.7 mmol•L^{-1}). No change in body composition was observed in either group throughout the trial (Figure 7.3.4 A – C).

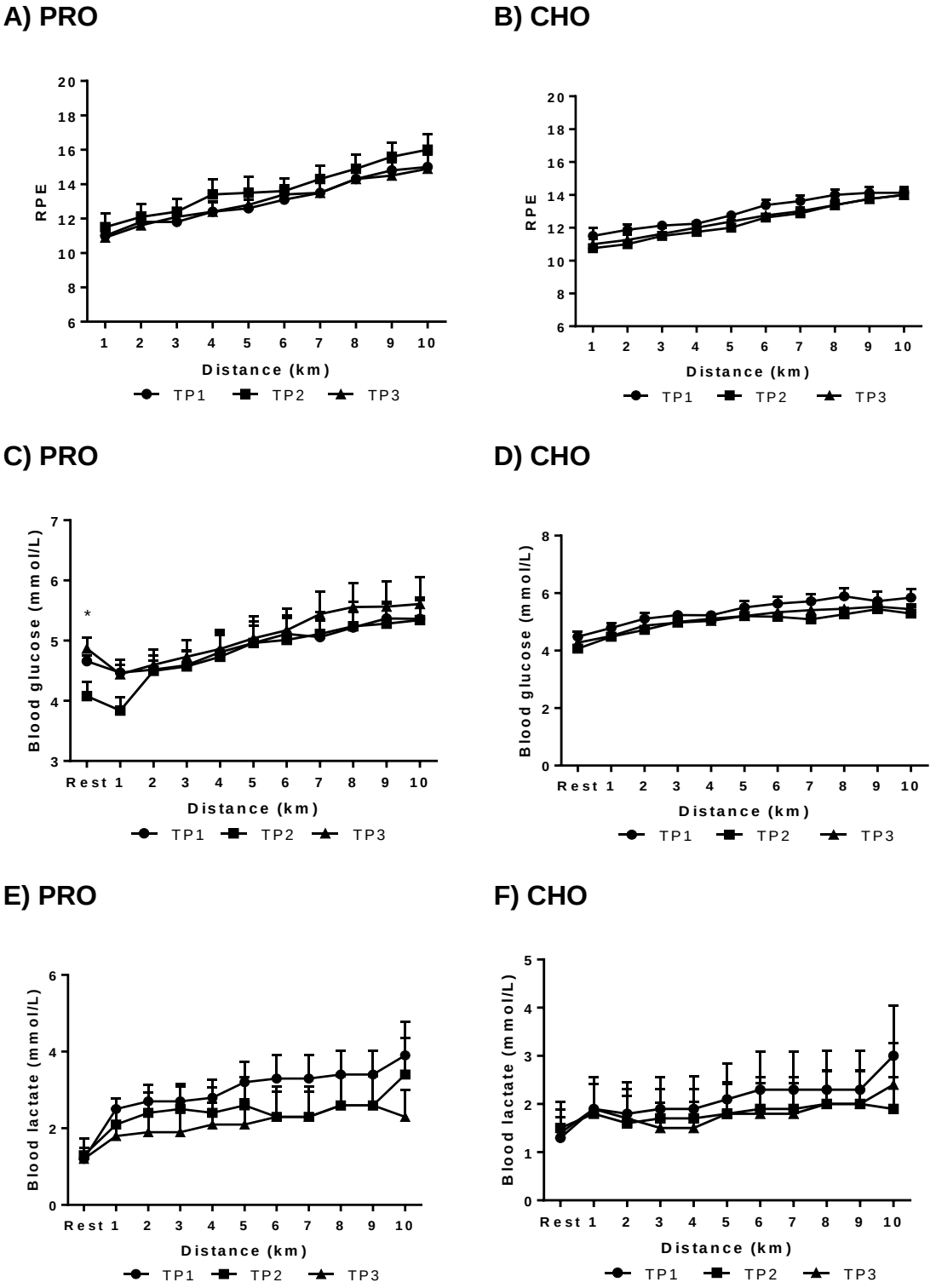


Figure 7.3.5. Post intervention blood lactate, blood glucose and rating of perceived exertion during a 10 km treadmill run at 75 % $\dot{V}O_2$max.

*Impact of 7-days habitual diet (TP1 and TP3) and 7-days intervention diet (high carbohydrate (CHO) or a high protein (PRO)) (TP2) in combination with endurance training on RPE (**A** and **B**), blood glucose (**C** and **D**) and blood lactate (**E** and **F**) * = TP2 significantly different from TP1 and TP3 ($p < 0.05$) (mean ± SEM).*

Table 7.3.3. Aerobic and anaerobic contribution towards energy production for the 10 km steady state run.

	Week 1		**Week 2**		**Week 3**	
	Aerobic (%)	Anaerobic (%)	Aerobic (%)	Anaerobic (%)	Aerobic (%)	Anaerobic (%)
PRO	98.9 ± 0.3	1.1 ± 0.3	98.8 ± 0.3	1.2 ± 0.3	98.9 ± 0.2	1.1 ± 0.2
CHO	98.9 ± 0.2	1.1 ± 0.2	99.0 ± 0.2	1.0 ± 0.2	99.0 ± 0.2	1.0 ± 0.2

Table 7.3.4. Aerobic and anaerobic contribution towards energy production for the MSE effort.

	Week 1		**Week 2**		**Week 3**	
	Aerobic (%)	Anaerobic (%)	Aerobic (%) §	Anaerobic (%) §	Aerobic (%) §	Anaerobic (%) §
PRO	74.2 ± 7.3	25.8 ± 7.3	65.4 ± 8.8*¥	34.6 ± 8.8*¥	74.9 ± 6.3	25.1 ± 6.3
CHO	79.5 ± 3.6	20.5 ± 3.6	82.9 ± 2.8*	17.1 ± 2.8*	82.1 ± 3.2	17.9 ± 3.2

*** =** *significant difference from equivalent time point 1 result (p < 0.05)*

¥ = significant difference from equivalent time point 1 result (p < 0.05)

§ = significant group interaction at the time point (p < 0.02)

No significant time point or group interactions were observed in either the aerobic or anaerobic contribution to energy expenditure for the 10 km steady state run (Table 7.3.3). The dietary intervention resulted in significant changes in energy contribution of the MaxSE. Following the high protein diet (week 2), a significant ($p < 0.05$) reduction in aerobic energy contribution and a significant increase in anaerobic contribution was observed relative to week 1 and week 3, no difference was observed between weeks 1 and 3 (Table 7.3.4). Subsequent to the high carbohydrate intervention diet (TP2) a significant increase in aerobic energy contribution and a significant decrease in energy contribution were observed relative to week 1 in the TTE trial, no other differences were observed in the carbohydrate group. No group interaction was observed ($p < 0.05$) at week 1; however the aerobic and anaerobic contribution towards energy expenditure were significantly different between groups at weeks 2 and 3, with a greater aerobic contribution subsequent to the high carbohydrate intervention.

7.4 Discussion

With regards to the research hypotheses the following can be concluded:

i) As demonstrated from the analysis of muscle-biopsies and in rejection of the **H_0**, a 7-day high protein low carbohydrate diet in combination with endurance training does not further enhance the mRNA transcriptional response.
ii) In rejection of the **H_0**, a 7-day high protein low carbohydrate diet in combination with endurance training resulted in a significant change in sub-maximal substrate utilisation. A 101% increase in fat oxidation and 27% decrease in carbohydrate oxidation were observed during a 10km steady state run. No change was seen in the oxidation rates following a high carbohydrate diet.
iii) In rejection of the **H_0**, 7-days consuming a high protein low carbohydrate diet in combination with endurance training resulted in a 23% reduction in the time to exhaustion trial.
iv) Adaptations that occurred subsequent to consuming a high protein diet, in combination with endurance training, reverted back to pre intervention levels once habitual diet was consumed again. Therefore the **H_0** was accepted, no long-term adaptations were observed following the intervention diet.

The impact of an acute continuous high protein diet in combination with endurance running training on metabolic adaptation, substrate utilisation and exercise performance has previously not been investigated. As hypothesised, significant shifts in substrate utilisation were observed following a 7-day high protein low carbohydrate diet. However, and an important consideration, high intensity exercise performance was significantly reduced (as reported by a 23% reduction in the maximal sustainable effort trial). Contrary to this, no change in sub-maximal substrate utilisation was observed when consuming a high carbohydrate (HC) diet for 7-days. Based on the chosen *p* value ($p < 0.05$) the observed change in time to exhaustion (TTE) in the high carbohydrate group was not significant ($p = 0.051$) however a trend towards improvement was reported (~6.5% increase). The change in resting expression of mRNA transcriptional proteins responsible for mitochondrial biogenesis and fatty acid

oxidation was not as hypothesised. A significant ($p < 0.05$) increase in AMPK and PPAR expression was observed in the CHO group, however no change in PGC-1α mRNA expression was observed in either intervention group. Additionally this is the first study to observe the effect of returning back to habitual diet subsequent to a low carbohydrate intervention, demonstrating that all performance measures in both groups returned to pre intervention levels. Interestingly however, the significant increase in AMPK and PPAR mRNA expression in the HC group was maintained once habitual diet was returned.

A recent review from Burke (2015) re-examined the literature for low carbohydrate (high fat) diets and sports performance, highlighting twelve studies, all of which assessed endurance performance using a cycling protocol, as such this was the first study to investigate the impact of acute continuous low carbohydrate feedings in well trained runners.

Endurance exercise is a potent stimulator of PGC-1α via the AMPK-SIRT1 pathway (Norrbom et al., 2004) and increases in PGC-1α expression have been observed following a single bout of endurance exercise (Perry et al., 2010). This response can be further amplified when exercising in a low glycogen state (Psilander et al., 2013). Furthermore it has been demonstrated *in vitro* (Canto et al., 2010) and *in vivo* (Draznin et al., 2012) that reduced glucose and/or nutrient availability promote increased PGC-1α, AMPK and SIRT1 mRNA expression. In untrained individuals over a 14-day training period Perry et al. (2010) found a transient post exercise response in PGC-1α expression, observing a 10-fold increase in PGC-1α mRNA expression subsequent to the first bout of endurance cycling which was reduced to a 4-fold increase after the seventh training session. In addition, with adequate glycogen availability Psilander et al. (2013) found a 2.5-fold increase in PGC-1α mRNA expression following a single bout of endurance cycling in well trained cyclists ($\dot{V}O_2max = 65.4 \pm 0.9$ mL•kg^{-1}•min^{-1}), and with the addition of glycogen restriction to the endurance session Psilander et al. (2013) observed an 8.1-fold increase in expression; a similar amplitude to the response observed in untrained individuals from Perry et al. (2010). These data sets suggest that the transcriptional adaptive response is blunted when skeletal muscle becomes accustomed to the exercise stress, and increasing the metabolic stress by restricting glycogen availability may result in enhanced post exercise transcriptional

adaptation. However the results from this experimental Chapter provide contradictory evidence; demonstrating reduced carbohydrate intake in combination with endurance training did not further increase resting PGC-1α mRNA expression.

As discussed above, previous literature has demonstrated that acute exercise with low glycogen availability increased metabolic stress and subsequent mRNA expression of transcriptional markers of mitochondrial biogenesis (Palacios et al., 2009, Psilander et al., 2013). However the current study appears to suggest that a high carbohydrate diet, in combination with endurance exercise, elicits the greatest changes in mRNA expression. A small but significant increase in resting AMPK (1.44 fold increase) and PPAR (1.32 fold increase) mRNA expression were observed following a continuous high carbohydrate diet for 7-days in combination with endurance running training. In the high protein group an increase in AMPK mRNA expression was observed following the intervention, however this returned to pre intervention levels once habitual diet was resumed (Figure 7.3.1). No significant time point difference in training distance or running speed was observed in either group. However, it is important to consider that a (non-significant) 0.9 km•h^{-1} reduction in average running speed between TP1 and TP2 (intervention week) in the high protein group was observed. This reduction in running speed was observed in seven of the eight participants (range -0.1 to -2.8 km•h^{-1}) in this group, suggesting that the reduction in dietary carbohydrate and/or the increase in dietary protein impacted the participant's ability to maintain training intensity.

It has been suggested that training low carbohydrate availability reduces exercise intensity and subsequently the quality of endurance training sessions (Burke et al., 2002). As previously stated, it is assumed that a greater metabolic stress during endurance exercise enhances mRNA expression. Both endurance exercise and carbohydrate restriction are potent stimulators of mRNA expression (Psilander et al., 2013). The high protein diet increased metabolic stress due to restricted carbohydrate availability; however training intensity was subsequently impacted and reduced. Baar et al. (2002) demonstrated a reduction in post exercise mRNA expression as skeletal muscle became accustomed to metabolic stress. Although non-significant, the results in this experimental Chapter suggest greater adaptation in the group that was able to maintain training intensity. High intensity running performance was decreased in the

high protein group who exhibited a 0.9 km•h^{-1} reduction in training speed subsequent to the dietary intervention, subsequent TTE performance was significantly impacted also (23.3% decrease). Taking these findings into account it could be hypothesised that maintaining training intensity is the key stressor to stimulate the AMPK/SIRT1 – PGC-1α metabolic pathway during repeated days of endurance running training. This may explain the trend towards a difference in post interventional mRNA expression between the two groups. Although restricting carbohydrate intake in the high protein group increased metabolic stress, the hypothesised increased training adaptation may have been compromised by the reduction in training intensity.

Furthermore, the results provide novel insight in to the long term adaptations of endurance training on metabolic markers of mitochondrial biogenesis at rest. The observed post intervention changes in AMPK and PPAR mRNA expression (1.44 and 1.32 fold increase, respectively) in the high carbohydrate group were maintained 7-days following a return to habitual diet (1.57 fold and 1.29 fold increase, respectively), where as the only cellular metabolic adaptation observed subsequent to the high protein intervention (1.4 fold increase in AMPK expression) returned to pre intervention levels. This suggests training intensity is a dominant stimulus in post endurance training transcriptional gene response.

An important consideration is that post exercise muscle biopsies were not collected. However the results from the previous experimental (Chapter 6) in this thesis, along with Draznin et al. (2012) demonstrated that mRNA expression is sensitive to dietary manipulation at rest and Baar et al. (2002) found that PGC-1 protein responds similar to mRNA expression, thus an assumption can be made that an increase in mRNA expression is accompanied by increased protein content.

Training in a glycogen depleted state, to enhance fat metabolism and reduce carbohydrate oxidation is considered a positive adaptation for endurance performance as it can spare muscle glycogen utilisation and prolong endurance exercise (Goedecke et al., 1999). The majority of research in this field manipulates carbohydrate intake by increasing dietary fat (Hawley and Morton, 2014), whereas it was achieved by increasing dietary protein intake in this study. Muscle glycogen was not measured, however seminal work from Hultman and Bergstrom (1967)

demonstrated that consuming a very low carbohydrate diet for 7-days without exercising resulted in a 32% reduction in muscle glycogen. Thus it is fair to assume that the high protein intervention, in combination with endurance training, resulted in significant reductions in muscle glycogen. The significant reduction in resting blood glucose, and increase in fat oxidation, during the sub-maximal trial in the high protein group corroborate this further.

Short-term (~2-4 days) exposure to a low carbohydrate diet reduces exercise capacity by depleting liver and muscle stores of glycogen without producing a compensatory increase in fat oxidation (Pitsiladis and Maughan, 1999); whereas longer-term (≥ 5 days) adherence can cause a range of adaptations which enhance the breakdown, transportation and oxidation of fat in skeletal muscle (Burke, 2015). The results from this study demonstrated that a 7-day high protein diet, in combination with endurance training, had a profound impact on substrate utilisation during a 75% $\dot{V}O_2$max sub maximal 10km steady-state run (Figure 7.3.2; $p < 0.0001$. A 101% increase in fat oxidation (0.29 ± 0.05 to 0.59 ± 0.05 g•min^{-1}) and a 27% decrease (2.96 ± 0.18 to 2.15 ± 0.19 g•min^{-1}) in carbohydrate oxidation was observed. No time point changes in substrate utilisation were apparent in the high carbohydrate group. Although not reported, it is important to acknowledge to potential impact of increased dietary protein on substrate utilisation during exercise. Protein has a respiratory quotient of ~0.81 (Jeukendrup and Wallis, 2005), and increasing the protein content of the diet has been demonstrated to increase protein metabolism (Garlick et al., 1999), however as protein is not fully oxidised *in vivo* it is complex to quantify the effect. Additionally the equations developed to calculate substrate oxidation during exercise (which were used in this Chapter) assume negligible contributions of protein oxidation (Jeukendrup and Wallis, 2005).

The observed increase in fat oxidation subsequent to the high protein intervention, coupled with a significant increase in AMPK mRNA expression (transcriptional regulator linked to increased fatty acid oxidation), would suggest a significant metabolic adaptation had occurred. However upon return to habitual diet, substrate utilisation and AMPK expression in the high protein intervention returned to pre intervention levels. This suggests that observed changes measured in response to high protein feedings are not the initiation of a lasting adaptation, rather an acute

temporal response to meet the energy demands of the exercise due to the reduction in energy availability.

Lambert et al. (1994) found similar profound increases in fat oxidation during sub-maximal cycling, demonstrating that a 14-day high fat low carbohydrate diet increased fat oxidation by 87%, relative to a high carbohydrate low fat diet. Furthermore, Lambert et al. (1994) observed a fat oxidation rate of 0.6 g•min^{-1} subsequent to a 14-day HF (Table 7.4.1) diet, almost identical to the fat oxidation rate during the 10km SS run of 0.59 g•min^{-1} in this trial. following either 14-days high fat or high carbohydrate diet Lambert et al. (1994) reported no significant difference in TTE between groups. However caution should be taken with this observation. TTE in the high fat group was 8.3 ± 2.3 minutes compared to 12.5 ± 3.8 minutes (mean ± SEM) in the high carbohydrate group. The standard error of mean in the TTE was very large and with a small sample size (*N* = 5) it is no surprise that this did not prove statistically significant. Practically, a 33.6% reduction in TTE at 90% $\dot{V}O_2$max was observed following 2-weeks high fat diet, which in terms of performance is profound.

Table 7.4.1. Effects of dietary manipulation on sub-maximal (50% $\dot{V}$O2max) (**A**) and TTE (90% $\dot{V}$O2max) (**B**) cycling performance subsequent to 14-days high fat, low carbohydrate diet
(Lambert et al., 1994)

	HIGH-FAT (n=5)		HIGH-CHO (n=5)	
	mean	SEM	mean	SEM
Time to exhaustion (min)	79.7	7.6	42.5**	6.8
Heart rate (beats·min^{-1})	142	7	143	8
Oxygen uptake (l·min^{-1})	2.28	0.06	2.38	0.05
Ventilation (l·min^{-1})	50.4	1.5	59.2*	0.8
CHO oxidation (g·min^{-1})	1.41	0.70	2.23	0.40
Fat oxidation (g·min^{-1})	0.60	0.12	0.32	0.07
Respiratory exchange ratio	0.87	0.03	0.92*	0.02

A

	HIGH-FAT (n=5)		HIGH-CHO (n=5)	
	mean	SEM	mean	SEM
Time to exhaustion (min)	8.3	2.3	12.5	3.8
Heart rate (beats·min^{-1})	186	3	181	2
Oxygen uptake (l·min^{-1})	3.62	0.27	3.63	0.39
Ventilation (l·min^{-1})	120.9	5.9	122.7	6.3
Respiratory exchange ratio	1.07	0.04	1.15	0.03

B

The results from the current study demonstrated a significant reduction in high intensity exercise performance subsequent to a 7-day high protein diet, with a relative decrease of 23.3% in TTE at time point 2. In the high carbohydrate group TTE was not statistically (p = 0.051) difference, however a 6.5% increase in TTE was observed. The 6.5% increase may not be significant, but similar to Lambert et al. (1994) SD was large. The MaxSE trial used in this study had a CV of 5.6%, therefore it may be argued

that a 6.5% increase in TTE is meaningful. Additionally in middle and long distance events winning margins are often < 1%, thus a 6.5% increase could be seen as profound. It is important to consider that Lambert et al. (1994) utilised a different exercise modality, the exercise intensities were not matched and dietary manipulation was different durations. However with the trends in results from both studies being similar it may be suggested that high protein and high fat diets have similar effects on high intensity exercise performance and substrate utilisation, and a high protein diet is as effective as a high fat diet in increasing sub-maximal fat oxidation; however this needs to be explored further.

The high protein diet significantly altered the aerobic and anaerobic contribution of energy in the MaxSE trial. At TP1 an aerobic – anaerobic ratio of 2.9 (74.2 ± 7.3 and 25.8 ± 7.3) was recorded in the Max SE trial, this decreased to 1.9 (65.4 ± 8.8 and 34.6 ± 8.8) subsequent to the HP intervention, representative of a 12% reduction in aerobic contribution. Whereas the high carbohydrate group exhibited a significant increase in aerobic – anaerobic ratio at TP2 (3.8 vs. 4.8), a 4.2% increase in aerobic contribution. After the HP intervention TTE was significantly reduced (-23.3%), as was total energy expenditure. However as demonstrated in Figure 7.3.5 no pre - post time point difference in blood lactate were observed, thus it is fair to assume that the absolute anaerobic energy contribution to the MaxSE exercise was matched at both time points. Therefore the observed shift in aerobic/anaerobic ratio is due to a significant reduction in the absolute aerobic contribution to energy, which may be due to two mechanisms. Firstly, as the mean TTE was 23.3% longer at TP1 the energy cost for the effort was greater and as no time point difference in post TTE blood lactate post was observed the increase in energy expenditure must have been produced aerobically. Secondly, it may be that lactate production occurred faster at TP2 due to reduced glycogen availability, which increased metabolic acidosis and subsequently brought on exercise fatigue. Bergman and Brooks (1999) demonstrated no difference in blood lactate concentration following graded exercise in a fed and fasted state, thus it is fair to assume that the increase in TTE observed was due to increased glycogen availability to fuel aerobic energy production.

A further consideration from the study is participant perception and adherence of the diet. Following study completion the participants were informally asked how they felt

on the diet. These insights and conversation were not formally logged, which is a limitation, however it is important to report the discussions. None of the participants on the high carbohydrate diet had any complaints about lack of energy and were able to maintain consistent training. The participants on the high protein diet reported differently. One participant had to drop out due to the inability to undertake training whilst on the high protein diet. Another three participants reported that training felt harder and maintaining running speed was tough. A further three reported a change in toilet habits, with less regularity and a hardening of their stool. Similar considerations have been suggested following continuous high fat diets. Burke and Hawley (2002) reported a reduction in training capacity and increase in perceived effort in relation to the quality of high-intensity training, in athletes that consumed an acute high fat diet.

Following acute exercise, it has been demonstrated that restricted glycogen availability increases mRNA expression of transcriptional markers responsible for mitochondrial biogenesis. However no research has looked at the longer term impact on resting mRNA levels. The results from this study have demonstrated that training on a high protein diet did not results to long term (7-day) adaptation. More recently the method of periodising carbohydrate intake to match the needs of the training session (Burke, 2015), or possible sleeping with low carbohydrate availability (Marquet et al., 2016) has been investigated with some success (Marquet et al., 2016, Cochran et al., 2015) and it likely to be the most successful method. However the long term mechanistic response of skeletal muscle adaptation to an endurance training stimulus is not yet fully quantified and further research is required to understand the benefits of an increased acute transcriptional response and the impact this has on longer term adaptation.

7.5 Conclusion

In conclusion, this is the first investigation to determine the effects of a continuous high protein diet, combined with endurance training, on cellular adaptation and both sub-maximal and maximal exercise performance in runners. The results demonstrated that 7-days exposure to a high protein low carbohydrate diet resulted in significant increases in fat oxidation during sub maximal exercise; despite this a significant

reduction in TTE was observed. No significant changes were observed in the high carbohydrate diet in any of the performance measures. All changes in the sub-maximal performance markers observed in the high protein group were returned to pre intervention levels once habitual diet was reverted for 7-days. In addition the results demonstrated that a high carbohydrate diet in combination with endurance exercise resulted in a greater transcriptional mRNA response in upstream markers responsible for mitochondrial biogenesis which remained elevated 7-days after returning to habitual diet.

The results also demonstrated that the increases in sub-maximal fat oxidation subsequent to the high protein diet are of similar amplitude to that of high fat diet's cited in previous literature, suggesting that carbohydrate restriction is responsible for the increase in fat oxidation – not increases in fat or protein intake.

Limitations:

- Training was not prescribed. The aim of the study was to investigate the impact of the diet, not training stimulus. As such the participants were required to maintain current training load and replicate sessions. Positively, this study design provided confidence that all observed changes were due to the diet intervention. The participants were relatively well matched for running performance; however there was quite a large deviation in total training volume.
- Muscle glycogen was not measured. It is evident in the literature that a low carbohydrate (<35%) in combination with endurance training will deplete muscle glycogen stores. With current techniques, the only accurate method of measuring muscle glycogen is via a biopsy. The micro muscle-biopsy technique collected enough tissue for mRNA assessment, however this was not enough to measure muscle glycogen also.
- The study targeted a specific set of adaptive markers, with limited metabolite and hormonal markers. To develop a wider picture on the whole body impact of such diets on health a performance research is required encompassing different markers.

- The transcriptional mRNA genes analysed are upstream markers of mitochondrial biogenesis. To truly understand the long term and actual adaptation direct markers of adaptation, such as mitochondrial numbers / density / respiration would be required for assessment.
- Post exercise gene expression or activity was not measured. The research question throughout the thesis was to investigate resting gene expression. Furthermore with study design as stated, it would have required a further 6 biopsies (total of 9 biopsies) throughout the trial which would have significantly increased participant burden and discomfort. That aside, this would provide further insight and would be a suggested direction for future research.

8 GENERAL DISCUSSION

8.1 Study Perspectives

A number of research issues have been explored and discussed through each experimental Chapter. The aim of this Chapter is to bring together the pertinent learning's from each Chapter to scope the context, application and conclusion from this thesis.

8.1.1 Using the metabolic typing questionnaire in research for individualised diet prescription.

Individuals may respond differently to dietary intake and a significant amount of research exists stating dietary intake has an impact on metabolic function (O'Sullivan et al., 2011). Numerous commentaries have suggested that tailoring a diet to individual needs will help promote optimal health. Much of the rationale for using the commercially available metabolic typing questionnaire as a tool to prescribe dietary individualisation were developed from the original work from Williams (1956) on biochemical individuality, stating all humans are fundamentally disparate. Two book publications (Walcott and Fahey, 2000, Kristal and Haig, 2002) highlight numerous cases in which the application of the metabolic typing diet has cured chronic disease, however limited literature exists on the application of the metabolic typing questionnaire as a tool for individualised dietary prescription and Chapter 5 of this thesis was the first to investigate the use metabolic typing questionnaire in a research setting. Due to the limited literature caution should be taken when drawing conclusions and, if relevant, more research is needed to fully understand its application. The basis of the metabolic typing diet is that people are fundamentally different and thrive on different diets, however in Chapter five 94.3% of the participants (N = 88) were identified as the same mixed metabolic type and would subsequently be prescribed the same diet, thus it may be assumed that the questionnaire is not able to identify the relevant information to differentiate metabolic individuality and prescribe diets accordingly. In further support of this conclusion, Walcott and Fahey (2000) and Kristal and Haig (2002) state that metabolic control is dominated by either the fast or slow oxidative system which the questionnaire is designed to highlight. Due to the different physiological attributes of endurance and power athletes, it was hypothesised that

metabolic dominance would also be different. However no relationship was observed between metabolic type and athletic attributes further suggesting that the metabolic typing questionnaire is not able to differentiate metabolic characteristics.

The principal that the results of questionnaire can accurately prescribe individualised diets to improve health and performance is appealing. However new techniques and knowledge such as genomics, metabolomics and nutriomics has yet to provide a clear solution to provide dietary individualisation (Primrose et al., 2011), and without fully comprehending the impact of dietary intake on metabolism more evidence and research is needed before confidently using a tool to prescribe individualised diets. It is highly unlikely this tool will be the metabolic typing questionnaire.

8.1.2 Nutrition knowledge and dietary intake in UK athlete

Nutrition intake plays an essential role in human health and as alluded to in previous Chapters dietary intake in athletes is commonly reported as inadequate. Nutrition knowledge refers to knowledge of concepts and processes related to nutrition and health including knowledge of diet and health (Miller and Cassady, 2015) and it has been suggested that poor nutrition knowledge may be a contributing factor towards inadequate intake (Heaney et al., 2011). This relationship, however, appears inconsistent and it is likely that multiple factors may modify diet intake (Worsley, 2002). A suggested potential justification for this may be the poor quantification of nutrition knowledge. A range of nutrition knowledge questionnaires have been developed, however each questionnaire is population specific and validity is lost if utilised outside of the initial target population (Parmenter and Wardle, 1999). The processes completed in experimental Chapter 4 satisfy statistical validation and reliability measures and provide a new valid and reliable tool to measure nutrition knowledge in UK athletes (Appendix Four).

The results of the NKQA in Chapter 5 demonstrated that UK track and field athletes have greater nutrition knowledge than a non-athletic control group (60.4% vs. 48.6%) and the nutrition knowledge of the athletes were in line with previous literature from different athletic populations (Spendlove et al., 2012, Heaney et al., 2011). A

systematic review of nutrition knowledge in athletes concluded a weak positive association between nutrition knowledge and diet quality (r = 0.44) (Heaney et al., 2011), however the evidence is equivocal. This was the first study to investigate this relationship in UK track and field athletes relative to a non-athletic control population. Both athletic groups scored significantly higher on the NKQA and DQI compared to the control group however no individual association between nutrition knowledge and diet quality was apparent.

Corroborating previous literature, the results from Chapter 5 suggest that the quality of dietary intake is more complex than just enhancing education. UK track and field athlete were found to have better nutrition knowledge and higher dietary quality than non-athletes, however the dietary intake of the two athletic groups (power and endurance) were not different. With compelling literature demonstrating that endurance athletes and power athletes require different macronutrient ratios to fuel optimal performance this may be an area to focus education on (Tipton et al., 2007, Jeukendrup, 2011, Thomas et al., 2016). Interestingly a group difference in diet quality and nutrition knowledge was observed; the athletes demonstrated greater nutrition knowledge and diet quality than the control group, however no relationship was observed between diet quality and nutrition knowledge. This suggests that as a group, athletes' have greater nutrition knowledge than non-athletes, but nutrition knowledge is not a predictor diet quality. An important consideration is that diet quality is measured on micronutrient consumption and meeting / exceeding the RNI's is ranked as positive. Therefore as athletes had a higher calorie intake (i.e. athletes eat more) the reported improved diet quality in athletes may be consequential from the volume of food consumed. It appears that diet quality is more complex than just improving nutrition knowledge. A vast body of research has been completed over recent years and is yet to draw strong conclusions. It is clear the nutrition knowledge is a factor which impacts dietary choice, but the level of the impact is yet to be fully quantified. It appears that enhancing diet quality is more complex than just educating the athlete and it is probable that motivations for improvement are highly individual.

Interestingly as a footnote to this, the control group in this study reported a nutrition knowledge score of 48.6%. From a similar study in an Australian population, Spendlove et al. (2012) reported the non-educated control group had a nutrition

knowledge score of 63.1%. The validations of both questionnaires were robust and are deemed of equal difficulty due to similarity in score on dietetic trained individuals. Therefore it could be assumed that the Australian public have greater nutrition knowledge than an equivalent UK population. This may just be an observed association and further research is required to investigate this.

8.1.3 Metabolic adaptation at rest

Puigserver et al. (1998) first isolated PGC1-α demonstrating its key role in the regulation of mitochondrial processes and it is now well established that PGC1-α plays a crucial role in the regulation of mitochondrial biogenesis (Liang and Ward, 2006). PGC-1α phosphorylation occurs via several different pathways, both AMP-activated protein kinase (AMPK) and silent information regulator T1 (SIRT1) mediated deacetylation (Gurd et al., 2009) have been demonstrated to regulate the expression of PGC-1α in human muscle (Ruderman et al., 2010). Both of which are stimulated by environmental stressors such as nutrient availability (Nemoto et al., 2005), calorie restriction (Itani et al., 2003) and endurance exercise (Psilander et al., 2013). Previously Draznin et al. (2012) demonstrated in non-exercising muscle that high fat feeding (carbohydrate restriction) and calorie depravation provide sufficient stimulus to increase resting PGC-1α mRNA expression (Draznin et al., 2012), which, as demonstrated by Baar et al. (2002) leads to as similar protein response. Although no group difference were observed in the findings of Chapter 6, when accounting for time pont alone, the results suggest similar response while consuming a hypocaloric high protein diet low carbohydrate diet. The results from Chapter 6 in this thesis demonstrated in untrained healthy males such dietary intervention provided a significant metabolic stress to results in a time point increase in resting AMPK and PGC-1α mRNA expression.

The observed increased AMPK and PGC-1α mRNA expression subsequent to the high protein low carbohydrate hypocaloric diet may have importance implications longer term health outcomes. The evidence supporting the relationship between increased PGC-1α and subsequent increases in mitochondrial biogenesis is well substantiated (Ruderman et al., 2010), as is the relationship between mitochondrial

abundance/density and increased energy metabolism (Rodgers et al., 2008). A major defect in type 2 diabetes and obesity can be defined as a lack of metabolic stability in which the body, tissue and cells are not responsive to normal nutrient or hormonal fluctuations. A clear example is insulin resistance in type 2 diabetes. Diet induced metabolic adaptation may provide favorable adaptation decreasing susceptibility of developing metabolic syndrome through the transcriptional pathway (Figure 8.1.1.), however increased protein intake may have adverse effects on metabolic function, in particular insulin sensitivity (Smith et al., 2016). A wider scope of research is required to understand the full body impact of such diets.

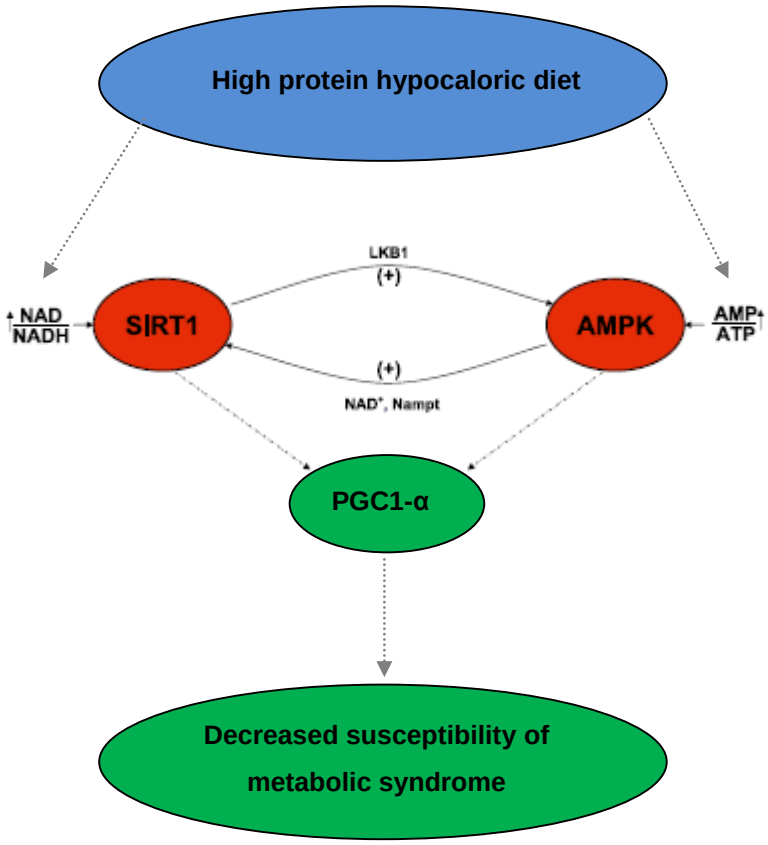


Figure 8.1.1. The relationship between nutrient manipulation and susceptibility to metabolic syndrome through the, the AMPK/SIRT1 – PGC-1α pathway

(Adapted from Ruderman et al. (2010) with results from Chapter 6).

In a systemic review Wycherley et al. (2012) suggested that during weight loss induced by a reduction in energy intake an increase in protein consumption provides additional

benefits including: enhancing the reductions in body mass and fat mass, mitigating reductions in lean mass, and mitigating reductions in RMR. With regards to body composition the data from Chapter 6 showed no difference in conditions that were matched for energy intake (EM and ER), difference were only observed in groups which were not balanced for energy intake (eucaloric vs. hypocaloric). Currently it is understood that a series of intracellular networks influence the molecular regulation of muscle protein turnover, and muscle mass regulation is a fine balance between muscle protein breakdown (MPB) and muscle protein synthesis (MPS) (Phillips and Van Loon, 2011). In a calorie restricted diet a high protein intake may have a sparing effect on lean mass loss. In overweight women Layman et al. (2003) reported greater fat mass loss and improved maintenance of fat free mass following a high protein diet (1.6 $g \cdot kg^{-1} \cdot day^{-1}$) relative to a high carbohydrate diet with protein intakes set to meet RDA (0.8 $g \cdot kg^{-1} \cdot day^{-1}$). The results from this study corroborate these findings, demonstrating no difference in lean mass loss between the hypocaloric high protein diet (2.5 $g \cdot kg^{-1} \cdot day^{-1}$ of protein) and the eucaloric high carbohydrate diet (2.5 $g \cdot kg^{-1} \cdot day^{-1}$ of protein). Interestingly, subsequent to the high protein hypocaloric diet an increase in AMPK mRNA expression was observed which is known to inhibit mTORC1 and subsequently down regulate MPS (Figure 8.1.2). This suggests that the observed maintenance of lean mass subsequent to a hypocaloric high protein diet is resultant of a decrease in protein breakdown rather than stimulating and increasing MPS. However, although increasing dietary protein intake to more than the RDA in a state of negative energy balance has been demonstrated to spare skeletal muscle metabolism a thorough understanding of the underlying molecular mechanisms is not fully developed. Such understanding is a requisite in the development of definitive nutritional strategies to mitigate the detrimental effects of negative energy balance (Carbone et al., 2012).

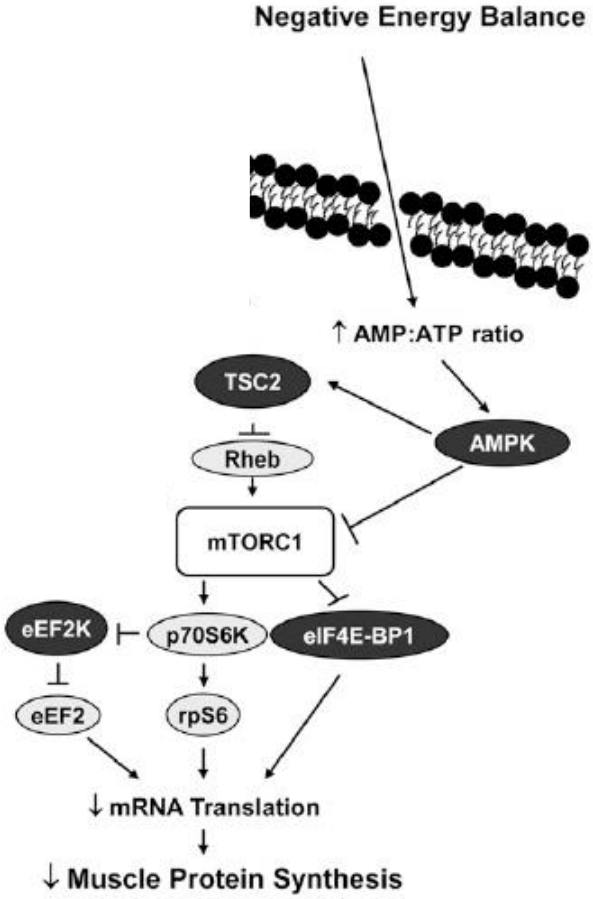


Figure 8.1.2. Role of negative energy balance and reduced growth factors on MPS (Adapted from Carbone et al. (2012)).

RMR accounts for ~65% of daily energy expenditure (Ravussin et al., 1986) and is strongly correlated with lean mass. Subsequent to a high protein diet Wycherley et al. (2012) attribute the observed increase in fat mass loss to a preservation of lean mass which was associated with increased RMR. Furthermore this increase in RMR (and calories burnt) via greater preservation of lean mass may also induce an increased net energy deficit over time that will promote fat metabolism. The hypocaloric high protein group lost significantly greater fat mass that the eucaloric high carbohydrate groups, with no difference observed between any other groups. However due to the acute nature of the dietary intervention no change in RMR was observed in any experimental group. Further applied research is required to assess the combination of negative energy balance and high protein dietary intake on cellular mechanisms contributing to skeletal muscle mass maintenance and RMR increases.

If fat mass loss is the goal of the dietary intervention the body composition and cellular adaptation results observed in Chapter 6 suggest that a high protein hypocaloric diet is more suitable for quick preferential changes in body composition. The increase in AMPK and PGC-1α suggest that over time skeletal muscle may have a greater capacity for aerobic metabolism as a result of increased mitochondrial biogenesis which may have a preferential impact on metabolic rate, insulin sensitivity and decreased susceptibility to metabolic syndrome. Individuals should consider the purpose of the intervention prior to automatically restricting carbohydrate and calorie intake (Figure 8.1.3), especially athletes. Further exploration into the impact of a high protein hypocaloric diet over a prolonged period of time is required to better understand the cellular, metabolic and transcriptional changes.

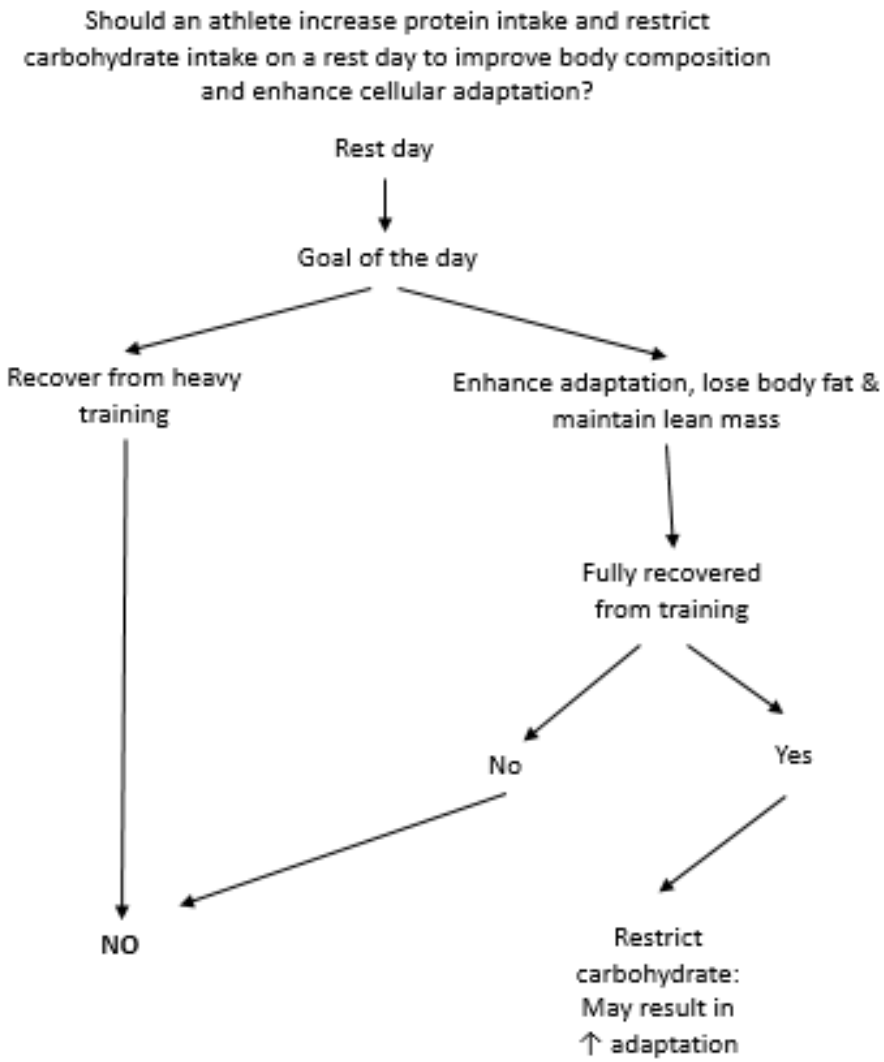


Figure 8.1.3. Flow diagram used to help athletes make an informed choice about whether to increase protein and restrict carbohydrate intake on a rest day.

8.1.4 High protein diet in combination with endurance training.

Increasing endurance exercise efficiency by increasing fat oxidation is understood to enhance endurance performance (Burke and Hawley, 2002). Consuming a high fat diet has been demonstrated to double fat oxidation during endurance exercise and consequently spare muscle glycogen (Lambert et al., 1994). The exact mechanisms for this are not defined, increased activity of β-hydroxyacyl-CoA-dehydrogenase, cartinine palmitoyl transferase I and decrease pyruvate dehydrogenase have been suggested to have a role (Helge and Kiens, 1997). Additionally increased inter muscular triglyceride stores have been detected after a period of a high fat diet, thus increasing the substrate pool may also influence fat oxidation (Jansson and Kaijser, 1982), also a high fat diet reduces the potential for glycogen oxidation (Stellingwerff et al., 2006). Importantly in some cases low carbohydrate (high fat) diets have been demonstrated to decrease high intensity endurance performance (O'Keeffe et al., 1989). Limited literature exists on the metabolic response subsequent to high protein feedings. After 7-day continuous high protein diet in combination with endurance exercise the results of Chapter 7 demonstrated a profound shift towards fat oxidation. Interestingly the amplitude of change was similar to that which was observed by Lambert et al. (1994) following a high fat diet in cyclists. Therefore an assumption may be made that an acute high protein diet increases sub-maximal fat oxidation to similar amplitude to that of a high fat diet. Suggesting that if the exercise goal is to increase fat oxidation and spare muscle glycogen utilisation, restricting carbohydrate intake is key; the method used to restrict carbohydrate intake is not important. The reduction in glycogen availability is the primary stimulus. However more research comparing a high protein diet to a high fat diet is needed to further corroborate this. Importantly though, that adaptations in substrate utilisation are not sustained following return to habitual dietary intake.

In untrained athletes Perry et al. (2010) demonstrated a ‘saw tooth’ effect in PGC-1α mRNA expression in response to endurance training. In a 14-day training study, subsequent to the first training sessions a >10 fold increase in mRNA expression was observed three hour post exercise, however by session seven (day 14) in the study

the response was attenuated to a 4-fold increase, however no change in resting mRNA was observed. Concurrently throughout the study PGC-1α protein content was measured, and subsequent to a steep rise a plateau and possible slight decrease in protein response was observed. This study from Perry et al. (2010) does not demonstrate longer term mRNA and protein response, however this data suggests that as untrained individuals become accustomed to the exercise, the training provided less metabolic stress and the amplitude of post exercise transcriptional response is dampened. It is well documented that glucose restriction increases PGC-1α expression; as such restricting glycogen availability in combination with the training stimulus has been demonstrated to increase metabolic stress and subsequently increase transcriptional response 4 times (Psilander et al., 2013).

Numerous studies have demonstrated that a single bout of endurance exercise in a glycogen depleted state increases post exercise PGC-1α expression to a greater extent that exercise with glycogen availability (Hawley and Morton, 2014). However the impact of continuous glycogen restriction in combination with endurance is less well established. The studies from Cochran et al. (2015) and Perry et al. (2010) in untrained participants demonstrated a positive adaptive response to endurance training with and without carbohydrate restriction. In Chapter 6, in untrained individuals at rest a similar increased adaptive response was observed subsequent to calorie and carbohydrate restriction. However well-trained individuals in combination with endurance training, continuous (7-day) glycogen restriction (high protein) did not further enhance metabolic adaptation, and potentially may have even blunted this response; the increased metabolic stress through glycogen restriction did not prove sufficient to impact the cellular adaptive response. Interestingly an increase in resting AMPK and PPAR mRNA expression was observed and maintained in the high carbohydrate diet; it may also be argued that the high carbohydrate groups were under less metabolic stress through dietary interventions. An important consideration in the results from Chapter 7 was that training speed was able to be maintained in the high carbohydrate groups – a 0.9 $km \cdot h^{-1}$ reduction was observed in the high protein groups. Considering previous literature and the results from Chapter 7 it could be concluded that at rest, in untrained muscle, increasing metabolic stress (hypocaloric low carbohydrate diet) is sufficient to stimulate the cellular adaptation, however in well-trained individuals this is not the case.

In Chapter 7 the athletes who consumed a continuous high protein diet for 7-days exhibited a mean reduction in training speed of 0.9 km•h^{-1}, however the athletes on the high carbohydrate diet were able to maintain training speed during the intervention. Therefore the high protein group exhibited an increase in metabolic stress by restricting glycogen availability and the high carbohydrate increased metabolic stress by maintaining training intensity. In terms of exercise performance TTE in the high carbohydrate group was significantly better; also the participants exhibited a more favourable gene response. Thus it may be postulated that in well-trained athletes, training intensity is the most important regulator of AMPK/SITR1 – PGC-1α metabolic pathway. The increase in metabolic stress by reducing glycogen availability in the high protein group was not enough to offset the reduction in training intensity.

8.2 Overall study perspectives

In Chapter 6 the high protein, hypocaloric group had a reduction in energy intake of ~1,000 kcal•day^{-1} (1,876 kcal) and in Chapter 7 the estimated mean additional energy expedited from running training was ~800 kcal•day^{-1} (45 mins – 60 mins running per day), the same macronutrient ratios were utilised in each Chapter. It was demonstrated in Chapter 6 that the reduction in calorie and carbohydrate intake provided sufficient metabolic stress to promote cellular adaptation, however in experimental Chapter 7, utilising the same dietary ratios the additional energy expended through running training did not enhance adaptation. Contrary to this, the high carbohydrate group who were able to maintain training intensity exhibited a preferential adaptive cellular response. Considering that in Chapter 6 no change was observed in the cellular adaptive at rest in hypocaloric high carbohydrate group, but the equivalent carbohydrate restricted (high protein) diet did, suggests that *in vivo* restricted carbohydrate intake is a more potent regulator of metabolic adaptation of mitochondrial biogenesis than calorie restriction at rest. However in well trained athletes and accompanied with running training, exercise intensity replaces energy availability as the dominant stimulator of metabolic adaptation, as such emphasis should be made on the maintenance of training intensity rather than carbohydrate and/or energy restriction for endurance athletes (Figure 8.2.1)

There seems to be a fine balance between fuel availability, increasing metabolic stress and maintaining exercise intensity to optimising training adaptation. The exact protocol to optimise this is yet to be defined. However, the greater understanding we have of the mechanistic processes involved in the adaptive response to training, the closer we will be to answering this question.

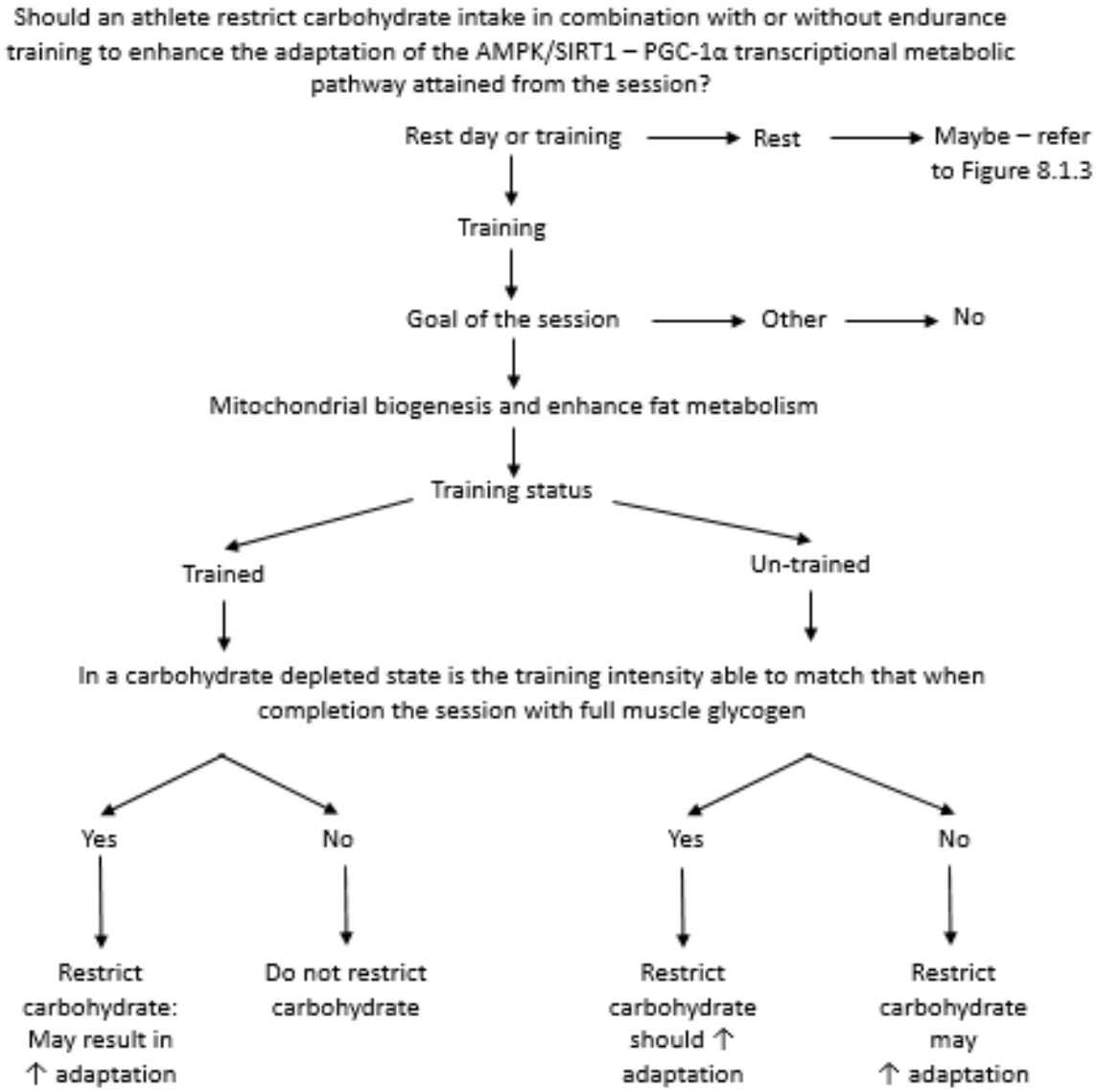


Figure 8.2.1. Flow diagram used to help make an informed choice about whether to choose to train on a low carbohydrate diet or not.

8.3 Application

- The questionnaire developed in Chapter 4 provides a tool for practitioners and athletes to measure nutritional knowledge, define gaps in nutrition knowledge, and for use in evidence based intervention and practice.
- The metabolic typing questionnaire is not able to differentiate metabolic function in healthy individuals and caution should be taken by those intending to implement it.
- The endurance and power athletes in the study consumed very similar diets. Literature is evident demonstrating that a higher protein intake may benefit power athletes and increased carbohydrate may benefit endurance athletes. Coaches, practitioners and athletes should consider this and assess for dietary adequacy to meet required training demands.
- Athletes possess sound nutrition knowledge; however education around macronutrient quantities present in foods may enhance knowledge – potentially improving practice.
- At rest, consuming a diet which restricts carbohydrate intake in a hypocaloric state promotes preferential metabolic adaptations commonly associated with endurance training. The implications of this Chapter suggest two key applications. Firstly, for a non-athletic individual aiming to lose weight, a high protein, hypocaloric diet results in preferential body composition changes, greater fat loss and less skeletal muscle loss. Secondly, this suggests that for an athlete, on a non-training day, restricting carbohydrate intake may promote preferential metabolic cellular adaptations that are associated with endurance training.
- Furthermore the results suggest that in well-trained runners it is imperative to maintain intensity during endurance training to promote preferential adaptation and performance. However as stated in previous literature, if training intensity is able to be maintained then restricting glycogen availability may result in greater adaptation.
- For athletes looking to enhance fat metabolism during endurance exercise, a high protein diet, in combination with endurance training, seems to promote similar increases in fat oxidation during sub-maximal running as high fat diets stated in previous literature. It has been documented that high fat diets can be

un-enjoyable to consume; as such a high protein diet which results in the same adaptation maybe a more enjoyable may and a preferable alternative.

8.4 Future research

The completion of this thesis has raised a number of questions that would benefit from future research to provide greater comprehension:

1. Dietary intake in UK track and field athletes was similar in both the speed and endurance cohorts. It is well documented that the different physiological demands of the events require differing nutrition strategies to optimise performance. Were the observed results specific to the assessed cohort, if not why are athletes not following guidelines?
2. What are the shorter and longer-term impacts of consuming a high protein hypocaloric diet? Are the same adaptations observed subsequent to a single day's dietary interventions? Are the observed adaptation further enhanced if the diet is maintained?
3. On a rest day, if an athlete consumed a high protein hypocaloric diet would this enhance adaptation?
4. Is the a 'sweet spot' where a small compromise training intensity and increased metabolic stress due to glycogen restriction results in a preferential adaptation – if so what is it?
5. What is the impact of consuming a high protein diet vs. high fat diet in combination with endurance training on metabolic and cellular response? Is an increased protein intake more effective than increased fat?
6. What is the longer-term impact of training with a high protein diet? Experimental Chapter 7 demonstrated no adaptive response to the high protein diet 7-days post intervention. However as with altitude training it takes approximately 4 weeks to elicit adaptation which lasts up to 30-days on returned to sea level. Would a similar adaptive response be observed if the dietary intervention in the current study was longer?
7. Given that post exercise biopsies were not taken and the exercise was completed in a glycogen depleted state, further work is warranted to identify if,

subsequent to a high protein diet, refuelling skeletal muscle glycogen pre performance test attenuates the observed decrease in high intensity exercise performance.

9 REFERENCES

AHTIAINEN, J. P., PAKARINEN, A., ALEN, M., KRAEMER, W. J. & HAKKINEN, K. 2003. Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *Eur J Appl Physiol,* 89, 555-63.

AMERICAN DIETETIC, A., DIETITIANS OF, C., AMERICAN COLLEGE OF SPORTS, M., RODRIGUEZ, N. R., DI MARCO, N. M. & LANGLEY, S. 2009. American College of Sports Medicine position stand. Nutrition and athletic performance. *Medicine and science in sports and exercise,* 41, 709-31.

ANDERSON, A. S., BELL, A., ADAMSON, A. & MOYNIHAN, P. 2002. A questionnaire assessment of nutrition knowledge--validity and reliability issues. *Public health nutrition,* 5, 497-503.

ANDERSON, A. S., UMAPATHY, D., PALUMBO, L. & PEARSON, D. W. M. 1988. Nutrition knowledge assessed by a structures questionnaire in a group of medical in-patients. *Journal of Human Nutrition and Dietetics,* 1, 39-46.

ARETA, J. L., BURKE, L. M., CAMERA, D. M., WEST, D. W., CRAWSHAY, S., MOORE, D. R., STELLINGWERFF, T., PHILLIPS, S. M., HAWLEY, J. A. & COFFEY, V. G. 2014. Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am J Physiol Endocrinol Metab,* 306, E989-97.

AXELSON, M. L. & BRINBERG, D. 1992. The measurement and conceptualization of nutrition knowledge. *Journal of Nutrition Education,* 24, 239-246.

BAAR, K., WENDE, A. R., JONES, T. E., MARISON, M., NOLTE, L. A., CHEN, M., KELLY, D. P. & HOLLOSZY, J. O. 2002. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J,* 16, 1879-86.

BALLMER, P. E., MCNURLAN, M. A., HULTER, H. N., ANDERSON, S. E., GARLICK, P. J. & KRAPF, R. 1995. Chronic metabolic acidosis decreases albumin synthesis and induces negative nitrogen balance in humans. *J Clin Invest,* 95, 39-45.

BARTLETT, J. D., HAWLEY, J. A. & MORTON, J. P. 2015. Carbohydrate availability and exercise training adaptation: too much of a good thing? *Eur J Sport Sci,* 15, 3-12.

BASIOTIS, P. P., WELSH, S. O., CRONIN, F. J., KELSAY, J. L. & MERTZ, W. 1987. Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence. *J Nutr,* 117, 1638-41.

BERGMAN, B. C. & BROOKS, G. A. 1999. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol (1985),* 86, 479-87.

BERGSTROM, J., HERMANSEN, L., HULTMAN, E. & SALTIN, B. 1967. Diet, muscle glycogen and physical performance. *Acta Physiol Scand,* 71, 140-50.

BINGHAM, S. A., GILL, C., WELCH, A., DAY, K., CASSIDY, A., KHAW, K. T., SNEYD, M. J., KEY, T. J., ROE, L. & DAY, N. E. 1994. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr,* 72, 619-43.

BODEN, G., SARGRAD, K., HOMKO, C., MOZZOLI, M. & STEIN, T. P. 2005. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin

resistance in obese patients with type 2 diabetes. *Ann Intern Med,* 142, 403-11.

BORG, G. 1970. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med,* 2, 92-8.

BURKE, L. & DEAKIN, V. 2010. *Clinical Sports Nutrition,* New South Wales; Australia, McGraw-Hill.

BURKE, L. M. 2015. Re-Examining High-Fat Diets for Sports Performance: Did We Call the 'Nail in the Coffin' Too Soon? *Sports Med,* 45 Suppl 1, S33-49.

BURKE, L. M., ANGUS, D. J., COX, G. R., CUMMINGS, N. K., FEBBRAIO, M. A., GAWTHORN, K., HAWLEY, J. A., MINEHAN, M., MARTIN, D. T. & HARGREAVES, M. 2000. Effect of fat adaptation and carbohydrate restoration on metabolism and performance during prolonged cycling. *J Appl Physiol (1985),* 89, 2413-21.

BURKE, L. M., COX, G. R., CULMMINGS, N. K. & DESBROW, B. 2001. Guidelines for daily carbohydrate intake: do athletes achieve them? *Sports medicine,* 31, 267-99.

BURKE, L. M. & HAWLEY, J. A. 2002. Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med Sci Sports Exerc,* 34, 1492-8.

BURKE, L. M., HAWLEY, J. A., ANGUS, D. J., COX, G. R., CLARK, S. A., CUMMINGS, N. K., DESBROW, B. & HARGREAVES, M. 2002. Adaptations to short-term high-fat diet persist during exercise despite high carbohydrate availability. *Med Sci Sports Exerc,* 34, 83-91.

BURKE, L. M., MILLET, G. & TARNOPOLSKY, M. A. 2007. Nutrition for distance events. *J Sports Sci,* 25 Suppl 1, S29-38.

CANTO, C., JIANG, L. Q., DESHMUKH, A. S., MATAKI, C., COSTE, A., LAGOUGE, M., ZIERATH, J. R. & AUWERX, J. 2010. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab,* 11, 213-9.

CARBONE, J. W., MCCLUNG, J. P. & PASIAKOS, S. M. 2012. Skeletal muscle responses to negative energy balance: effects of dietary protein. *Adv Nutr,* 3, 119-26.

CAREY, A. L., STAUDACHER, H. M., CUMMINGS, N. K., STEPTO, N. K., NIKOLOPOULOS, V., BURKE, L. M. & HAWLEY, J. A. 2001. Effects of fat adaptation and carbohydrate restoration on prolonged endurance exercise. *J Appl Physiol (1985),* 91, 115-22.

CHAPMAN, R. F., LAYMON STICKFORD, A. S., LUNDBY, C. & LEVINE, B. D. 2014. Timing of return from altitude training for optimal sea level performance. *J Appl Physiol (1985),* 116, 837-43.

CHO, M. & FRYER, B. A. 1974. What foods do physical education majors and basic nutrition students recommend for athletes? *J Am Diet Assoc,* 65, 541-4.

COCHRAN, A. J., MYSLIK, F., MACINNIS, M. J., PERCIVAL, M. E., BISHOP, D., TARNOPOLSKY, M. A. & GIBALA, M. J. 2015. Manipulating Carbohydrate Availability Between Twice-Daily Sessions of High-Intensity Interval Training Over 2 Weeks Improves Time-Trial Performance. *Int J Sport Nutr Exerc Metab,* 25, 463-70.

COHEN, H. Y., MILLER, C., BITTERMAN, K. J., WALL, N. R., HEKKING, B., KESSLER, B., HOWITZ, K. T., GOROSPE, M., DE CABO, R. & SINCLAIR, D.

A. 2004. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science,* 305, 390-2.

COTUGNA, N., SUBAR, A. F., HEIMENDINGER, J. & KAHLE, L. 1992. Nutrition and cancer prevention knowledge, beliefs, attitudes, and practices: the 1987 National Health Interview Survey. *J Am Diet Assoc,* 92, 963-8.

COYLE, E. F., COGGAN, A. R., HEMMERT, M. K. & IVY, J. L. 1986. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol (1985),* 61, 165-72.

CRONBACH, L. J. 1951. Coefficent alpha and the internal structure of tests. *Psychometrika,* 16, 297-234.

CUPISTI, A., D'ALESSANDRO, C., CASTROGIOVANNI, S., BARALE, A. & MORELLI, E. 2002. Nutrition knowledge and dietary composition in Italian adolescent female athletes and non-athletes. *Int J Sport Nutr Exerc Metab,* 12, 207-19.

DALLONGEVILLE, J., MARECAUX, N., COTTEL, D., BINGHAM, A. & AMOUYEL, P. 2001. Association between nutrition knowledge and nutritional intake in middle-aged men from Northern France. *Public Health Nutr,* 4, 27-33.

DE FEYTER, H. M., LENAERS, E., HOUTEN, S. M., SCHRAUWEN, P., HESSELINK, M. K., WANDERS, R. J., NICOLAY, K. & PROMPERS, J. J. 2008. Increased intramyocellular lipid content but normal skeletal muscle mitochondrial oxidative capacity throughout the pathogenesis of type 2 diabetes. *FASEB J,* 22, 3947-55.

DELGUERCIO, L. R., FEINS, N. R., COHN, J. D., COOMARASWAMY, R. P., WOLLMAN, S. B. & STATE, D. 1965. Comparison of Blood Flow during External and Internal Cardiac Massage in Man. *Circulation,* 31, SUPPL 1:171-80.

DOUGLAS, P. D. & DOUGLAS, J. G. 1984. Nutrition knowledge and food practices of high school athletes. *J Am Diet Assoc,* 84, 1198-202.

DRAZNIN, B., WANG, C., ADOCHIO, R., LEITNER, J. W. & CORNIER, M. A. 2012. Effect of dietary macronutrient composition on AMPK and SIRT1 expression and activity in human skeletal muscle. *Horm Metab Res,* 44, 650-5.

FIELD, A. P. 2013. *Discovering statistics using IBM SPSS Statistics,* London, Sage publications.

FINEGOLD, S. M., ATTEBERY, H. R. & SUTTER, V. L. 1974. Effect of diet on human fecal flora: comparison of Japanese and American diets. *Am J Clin Nutr,* 27, 1456-69.

FORSLUND, A. H., EL-KHOURY, A. E., OLSSON, R. M., SJODIN, A. M., HAMBRAEUS, L. & YOUNG, V. R. 1999. Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization. *Am J Physiol,* 276, E964-76.

FOUNDATION, B. N. 2015. Nutrition Requirements. *In:* FOUNDATION, B. N. (ed.). nutrition.org.uk.

FOWKE, J. H. & MATTHEWS, C. E. 2010. PSA and body composition by dual X-ray absorptiometry (DXA) in NHANES. *Prostate,* 70, 120-5.

FRIEDLANDER, A. L., BRAUN, B., POLLACK, M., MACDONALD, J. R., FULCO, C. S., MUZA, S. R., ROCK, P. B., HENDERSON, G. C., HORNING, M. A., BROOKS, G. A., HOFFMAN, A. R. & CYMERMAN, A. 2005. Three weeks of caloric restriction alters protein metabolism in normal-weight, young men. *Am J Physiol Endocrinol Metab,* 289, E446-55.

FUJII, N., HAYASHI, T., HIRSHMAN, M. F., SMITH, J. T., HABINOWSKI, S. A., KAIJSER, L., MU, J., LJUNGQVIST, O., BIRNBAUM, M. J., WITTERS, L. A., THORELL, A. & GOODYEAR, L. J. 2000. Exercise induces isoform-specific increase in 5'AMP-activated protein kinase activity in human skeletal muscle. *Biochem Biophys Res Commun,* 273, 1150-5.

FULCO, M., CEN, Y., ZHAO, P., HOFFMAN, E. P., MCBURNEY, M. W., SAUVE, A. A. & SARTORELLI, V. 2008. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev Cell,* 14, 661-73.

GAITANOS, G. C., WILLIAMS, C., BOOBIS, L. H. & BROOKS, S. 1993. Human muscle metabolism during intermittent maximal exercise. *J Appl Physiol (1985),* 75, 712-9.

GALESIC, M. & BOSNJAK, M. 2009. Effects of Questionnaire Length on Participation and Indicators of Response Quality in a Web Survey. *Public Opinion Quarterly,* 73, 349-360.

GANESHAN, K. & CHAWLA, A. 2014. Metabolic regulation of immune responses. *Annu Rev Immunol,* 32, 609-34.

GARLICK, P. J., MCNURLAN, M. A. & PATLAK, C. S. 1999. Adaptation of protein metabolism in relation to limits to high dietary protein intake. *Eur J Clin Nutr,* 53 Suppl 1, S34-43.

GIBALA, M. J., MCGEE, S. L., GARNHAM, A. P., HOWLETT, K. F., SNOW, R. J. & HARGREAVES, M. 2009. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol (1985),* 106, 929-34.

GLEESON, M. & BISHOP, N. C. 2000. Special feature for the Olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements. *Immunol Cell Biol,* 78, 554-61.

GOEDECKE, J. H., CHRISTIE, C., WILSON, G., DENNIS, S. C., NOAKES, T. D., HOPKINS, W. G. & LAMBERT, E. V. 1999. Metabolic adaptations to a high-fat diet in endurance cyclists. *Metabolism,* 48, 1509-17.

GREENHAFF, P. L., NEVILL, M. E., SODERLUND, K., BODIN, K., BOOBIS, L. H., WILLIAMS, C. & HULTMAN, E. 1994. The metabolic responses of human type I and II muscle fibres during maximal treadmill sprinting. *J Physiol,* 478 (Pt 1), 149-55.

GRUNDY, S. M. & DENKE, M. A. 1990. Dietary influences on serum lipids and lipoproteins. *J Lipid Res,* 31, 1149-72.

GURD, B. J., YOSHIDA, Y., LALLY, J., HOLLOWAY, G. P. & BONEN, A. 2009. The deacetylase enzyme SIRT1 is not associated with oxidative capacity in rat heart and skeletal muscle and its overexpression reduces mitochondrial biogenesis. *J Physiol,* 587, 1817-28.

HAINES, P. S., SIEGA-RIZ, A. M. & POPKIN, B. M. 1999. The Diet Quality Index revised: a measurement instrument for populations. *J Am Diet Assoc,* 99, 697-704.

HAMILTON, C. Y., SCHILLER, M. R. & BOYNE, L. 1994. Nutrition attitudes, practices, and views of selected Ohio WIC participants. *J Am Diet Assoc,* 94, 899-901.

HANSEN, A. K., FISCHER, C. P., PLOMGAARD, P., ANDERSEN, J. L., SALTIN, B. & PEDERSEN, B. K. 2005. Skeletal muscle adaptation: training twice every second day vs. training once daily. *J Appl Physiol (1985),* 98, 93-9.

HARNACK, L., BLOCK, G., SUBAR, A., LANE, S. & BRAND, R. 1997. Association of cancer prevention-related nutrition knowledge, beliefs, and attitudes to cancer prevention dietary behavior. *J Am Diet Assoc,* 97, 957-65.

HARRIS, J. A. & BENEDICT, F. G. 1918. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A,* 4, 370-3.

HARRISON, J., HOPKINS, W. G., MACFARLANE, D. J. & WORSLEY, A. 1991. Nutrition knowledge and dietary habits of elite and non-elite athletes. *Australian Journal of Nutrition and Dietetics,* 48, 124-127.

HAVEMANN, L., WEST, S. J., GOEDECKE, J. H., MACDONALD, I. A., ST CLAIR GIBSON, A., NOAKES, T. D. & LAMBERT, E. V. 2006. Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *J Appl Physiol (1985),* 100, 194-202.

HAWLEY, J. A., BROUNS, F. & JEUKENDRUP, A. 1998. Strategies to enhance fat utilisation during exercise. *Sports Med,* 25, 241-57.

HAWLEY, J. A. & MORTON, J. P. 2014. Ramping up the signal: promoting endurance training adaptation in skeletal muscle by nutritional manipulation. *Clin Exp Pharmacol Physiol,* 41, 608-13.

HAYOT, M., MICHAUD, A., KOECHLIN, C., CARON, M. A., LEBLANC, P., PREFAUT, C. & MALTAIS, F. 2005. Skeletal muscle microbiopsy: a validation study of a minimally invasive technique. *Eur Respir J,* 25, 431-40.

HEANEY, S., O'CONNOR, H., MICHAEL, S., GIFFORD, J. & NAUGHTON, G. 2011. Nutrition knowledge in athletes: a systematic review. *International journal of sport nutrition and exercise metabolism,* 21, 248-61.

HELGE, J. W. & KIENS, B. 1997. Muscle enzyme activity in humans: role of substrate availability and training. *Am J Physiol,* 272, R1620-4.

HENRY, C. J. 2005. Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutr,* 8, 1133-52.

HESHKA, S., YANG, M. U., WANG, J., BURT, P. & PI-SUNYER, F. X. 1990. Weight loss and change in resting metabolic rate. *Am J Clin Nutr,* 52, 981-6.

HILL, D. W. 1999. Energy system contributions in middle-distance running events. *J Sports Sci,* 17, 477-83.

HOLLOSZY, J. O. & COYLE, E. F. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol,* 56, 831-8.

HOLT, S. H., DELARGY, H. J., LAWTON, C. L. & BLUNDELL, J. E. 1999. The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. *Int J Food Sci Nutr,* 50, 13-28.

HULTMAN, E. & BERGSTROM, J. 1967. Muscle glycogen synthesis in relation to diet studied in normal subjects. *Acta Med Scand,* 182, 109-17.

HUSSAIN, T. A., MATHEW, T. C., DASHTI, A. A., ASFAR, S., AL-ZAID, N. & DASHTI, H. M. 2012. Effect of low-calorie versus low-carbohydrate ketogenic diet in type 2 diabetes. *Nutrition,* 28, 1016-21.

HUTBER, C. A., HARDIE, D. G. & WINDER, W. W. 1997. Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am J Physiol,* 272, E262-6.

ITANI, S. I., SAHA, A. K., KUROWSKI, T. G., COFFIN, H. R., TORNHEIM, K. & RUDERMAN, N. B. 2003. Glucose autoregulates its uptake in skeletal muscle: involvement of AMP-activated protein kinase. *Diabetes,* 52, 1635-40.

JAGER, S., HANDSCHIN, C., ST-PIERRE, J. & SPIEGELMAN, B. M. 2007. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A,* 104, 12017-22.

JANSSEN, I., HEYMSFIELD, S. B. & ROSS, R. 2002. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc,* 50, 889-96.

JANSSON, E. & KAIJSER, L. 1982. Effect of diet on the utilization of blood-borne and intramuscular substrates during exercise in man. *Acta Physiol Scand,* 115, 19-30.

JERLOCK, M., GASTON-JOHANSSON, F. & DANIELSON, E. 2005. Living with unexplained chest pain. *J Clin Nurs,* 14, 956-64.

JESSRI, M., JESSRI, M., RASHIDKHANI, B. & ZINN, C. 2010. Evaluation of Iranian college athletes' sport nutrition knowledge. *Int J Sport Nutr Exerc Metab,* 20, 257-63.

JEUKENDRUP, A. E. 2011. Nutrition for endurance sports: marathon, triathlon, and road cycling. *J Sports Sci,* 29 Suppl 1, S91-9.

JEUKENDRUP, A. E. & WALLIS, G. A. 2005. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med,* 26 Suppl 1, S28-37.

JOOST, H. G., GIBNEY, M. J., CASHMAN, K. D., GORMAN, U., HESKETH, J. E., MUELLER, M., VAN OMMEN, B., WILLIAMS, C. M. & MATHERS, J. C. 2007. Personalised nutrition: status and perspectives. *Br J Nutr,* 98, 26-31.

JORNAYVAZ, F. R. & SHULMAN, G. I. 2010. Regulation of mitochondrial biogenesis. *Essays Biochem,* 47, 69-84.

JOYNER, M. J., RUIZ, J. R. & LUCIA, A. 2011. The two-hour marathon: who and when? *J Appl Physiol (1985),* 110, 275-7.

KIM, J., WANG, Z., HEYMSFIELD, S. B., BAUMGARTNER, R. N. & GALLAGHER, D. 2002. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr,* 76, 378-83.

KLINE, P. 2007a. *The Handbook of Psychological Tes,* London, UK, Routledge.

KLINE, P. 2007b. *The Handbook of Psychological Testing,* London, UK, Routledge.

KRISTAL, H. J. & HAIG, J. M. 2002. *The Nutrition Solution: A Guide to Your Metabolic Type*, North Atlantic Books, California, United States of America.

LAMBERT, E. V., GOEDECKE, J. H., ZYLE, C., MURPHY, K., HAWLEY, J. A., DENNIS, S. C. & NOAKES, T. D. 2001. High-fat diet versus habitual diet prior to carbohydrate loading: effects of exercise metabolism and cycling performance. *Int J Sport Nutr Exerc Metab,* 11, 209-25.

LAMBERT, E. V., SPEECHLY, D. P., DENNIS, S. C. & NOAKES, T. D. 1994. Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. *Eur J Appl Physiol Occup Physiol,* 69, 287-93.

LARSEN, F. J., SCHIFFER, T. A., SAHLIN, K., EKBLOM, B., WEITZBERG, E. & LUNDBERG, J. O. 2011. Mitochondrial oxygen affinity predicts basal metabolic rate in humans. *FASEB J,* 25, 2843-52.

LAYMAN, D. K., BOILEAU, R. A., ERICKSON, D. J., PAINTER, J. E., SHIUE, H., SATHER, C. & CHRISTOU, D. D. 2003. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. *J Nutr,* 133, 411-7.

LEE, H. K., SONG, J. H., SHIN, C. S., PARK, D. J., PARK, K. S., LEE, K. U. & KOH, C. S. 1998. Decreased mitochondrial DNA content in peripheral blood precedes the development of non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract,* 42, 161-7.

LEONE, T. C., LEHMAN, J. J., FINCK, B. N., SCHAEFFER, P. J., WENDE, A. R., BOUDINA, S., COURTOIS, M., WOZNIAK, D. F., SAMBANDAM, N., BERNAL-MIZRACHI, C., CHEN, Z., HOLLOSZY, J. O., MEDEIROS, D. M., SCHMIDT, R. E., SAFFITZ, J. E., ABEL, E. D., SEMENKOVICH, C. F. & KELLY, D. P. 2005. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol,* 3, e101.

LIANG, H. & WARD, W. F. 2006. PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ,* 30, 145-51.

LIN, J., HANDSCHIN, C. & SPIEGELMAN, B. M. 2005. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab,* 1, 361-70.

MAGKOS, F. & YANNAKOULIA, M. 2003. Methodology of dietary assessment in athletes: concepts and pitfalls. *Curr Opin Clin Nutr Metab Care,* 6, 539-49.

MALIK, V. S. & HU, F. B. 2007. Popular weight-loss diets: from evidence to practice. *Nat Clin Pract Cardiovasc Med,* 4, 34-41.

MARQUET, L. A., BRISSWALTER, J., LOUIS, J., TIOLLIER, E., BURKE, L. M., HAWLEY, J. A. & HAUSSWIRTH, C. 2016. Enhanced Endurance Performance by Periodization of Carbohydrate Intake: "Sleep Low" Strategy. *Med Sci Sports Exerc,* 48, 663-72.

MARTIN, W. & MENTEL, M. 2010. The origin of mitochondria. *Nature Education,* 3, 58.

MASTORAKOS, G. & ZAPANTI, E. 2004. The hypothalamic-pituitary-adrenal axis in the neuroendocrine regulation of food intake and obesity: the role of corticotropin releasing hormone. *Nutr Neurosci,* 7, 271-80.

MCCANCE & WIDDOWSON. 2015. *Composition of foods integrated dataset (CoFID)* [Online]. https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid. [Accessed 28 August 2015].

MEIRHAEGHE, A., CROWLEY, V., LENAGHAN, C., LELLIOTT, C., GREEN, K., STEWART, A., HART, K., SCHINNER, S., SETHI, J. K., YEO, G., BRAND, M. D., CORTRIGHT, R. N., O'RAHILLY, S., MONTAGUE, C. & VIDAL-PUIG, A. J. 2003. Characterization of the human, mouse and rat PGC1 beta (peroxisome-proliferator-activated receptor-gamma co-activator 1 beta) gene in vitro and in vivo. *Biochem J,* 373, 155-65.

MEISENBERG, G. & WILLIAMS, A. 2008. Are acquiescent and extreme response styles related to low intelligence and education. *Personality and Individual Differences*, 1539-1550.

MERTZ, W., TSUI, J. C., JUDD, J. T., REISER, S., HALLFRISCH, J., MORRIS, E. R., STEELE, P. D. & LASHLEY, E. 1991. What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. *Am J Clin Nutr,* 54, 291-5.

MILLER, L. M. & CASSADY, D. L. 2015. The effects of nutrition knowledge on food label use. A review of the literature. *Appetite,* 92, 207-16.

MILLER, W. C., BRYCE, G. R. & CONLEE, R. K. 1984. Adaptations to a high-fat diet that increase exercise endurance in male rats. *J Appl Physiol Respir Environ Exerc Physiol,* 56, 78-83.

MISHRA, P. & CHAN, D. C. 2016. Metabolic regulation of mitochondrial dynamics. *J Cell Biol,* 212, 379-87.

MOOTHA, V. K., LINDGREN, C. M., ERIKSSON, K. F., SUBRAMANIAN, A., SIHAG, S., LEHAR, J., PUIGSERVER, P., CARLSSON, E., RIDDERSTRALE, M., LAURILA, E., HOUSTIS, N., DALY, M. J., PATTERSON, N., MESIROV, J. P., GOLUB, T. R., TAMAYO, P., SPIEGELMAN, B., LANDER, E. S., HIRSCHHORN, J. N., ALTSHULER, D. & GROOP, L. C. 2003. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet,* 34, 267-73.

MYERS, M. G., JR. & OLSON, D. P. 2012. Central nervous system control of metabolism. *Nature,* 491, 357-63.

NAKAZATO, K. & SONG, H. 2008. Increased oxidative properties of gastrocnemius in rats fed on a high-protein diet. *J Nutr Biochem,* 19, 26-32.

NASH, M. A., TORRADO, A. D., GREIFER, I., SPITZER, A. & EDELMANN, C. M., JR. 1972. Renal tubular acidosis in infants and children. Clinical course, response to treatment, and prognosis. *J Pediatr,* 80, 738-48.

NEMOTO, S., FERGUSSON, M. M. & FINKEL, T. 2005. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. *J Biol Chem,* 280, 16456-60.

NOAKES, T. 2004. Fat adaptation and prolonged exercise performance. *J Appl Physiol (1985),* 96, 1243; author reply 1243-4.

NORRBOM, J., SUNDBERG, C. J., AMELN, H., KRAUS, W. E., JANSSON, E. & GUSTAFSSON, T. 2004. PGC-1alpha mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J Appl Physiol (1985),* 96, 189-94.

O'KEEFFE, K. A., KEITH, R. E., WILSON, G. D. & BLESSING, D. L. 1989. Dietary carbohydrate intake and endurance exercise performance of trained female cyclists. *Nutrition Research,* 9, 819-830.

O'SULLIVAN, A., GIBNEY, M. J. & BRENNAN, L. 2011. Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies. *Am J Clin Nutr,* 93, 314-21.

PALACIOS, O. M., CARMONA, J. J., MICHAN, S., CHEN, K. Y., MANABE, Y., WARD, J. L., 3RD, GOODYEAR, L. J. & TONG, Q. 2009. Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. *Aging (Albany NY),* 1, 771-83.

PARMENTER, K. & WARDLE, J. 1999. Development of a general nutrition knowledge questionnaire for adults. *European journal of clinical nutrition,* 53, 298-308.

PATTI, M. E., BUTTE, A. J., CRUNKHORN, S., CUSI, K., BERRIA, R., KASHYAP, S., MIYAZAKI, Y., KOHANE, I., COSTELLO, M., SACCONE, R., LANDAKER, E. J., GOLDFINE, A. B., MUN, E., DEFRONZO, R., FINLAYSON, J., KAHN, C. R. & MANDARINO, L. J. 2003. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A,* 100, 8466-71.

PERRY, C. G., LALLY, J., HOLLOWAY, G. P., HEIGENHAUSER, G. J., BONEN, A. & SPRIET, L. L. 2010. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol,* 588, 4795-810.

PESSI, A. & FAYH, A. P. 2011. Evaluation of the Nutritional Knowledge of Professional Track and Field and Triathlon Athletes. *Revista Brasilerira de Medicina do Esporte,* 14.

PETERS, S. J. & LEBLANC, P. J. 2004. Metabolic aspects of low carbohydrate diets and exercise. *Nutr Metab (Lond),* 1, 7.

PETERSEN, K. F., DUFOUR, S., BEFROY, D., GARCIA, R. & SHULMAN, G. I. 2004. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med,* 350, 664-71.

PHILLIPS, S. M. 2006. Dietary protein for athletes: from requirements to metabolic advantage. *Appl Physiol Nutr Metab,* 31, 647-54.

PHILLIPS, S. M. & VAN LOON, L. J. 2011. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci,* 29 Suppl 1, S29-38.

PHINNEY, S. D., BISTRIAN, B. R., EVANS, W. J., GERVINO, E. & BLACKBURN, G. L. 1983. The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism,* 32, 769-76.

PIATTI, P. M., MONTI, F., FERMO, I., BARUFFALDI, L., NASSER, R., SANTAMBROGIO, G., LIBRENTI, M. C., GALLI-KIENLE, M., PONTIROLI, A. E. & POZZA, G. 1994. Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high-carbohydrate diet. *Metabolism,* 43, 1481-7.

PIROUZNIA, M. 2001. The association between nutrition knowledge and eating behavior in male and female adolescents in the US. *Int J Food Sci Nutr,* 52, 127-32.

PITSILADIS, Y. P. & MAUGHAN, R. J. 1999. The effects of exercise and diet manipulation on the capacity to perform prolonged exercise in the heat and in the cold in trained humans. *J Physiol,* 517 (Pt 3), 919-30.

PRIMROSE, S., DRAPER, J., ELSOM, R., KIRKPATRICK, V., MATHERS, J. C., SEAL, C., BECKMANN, M., HALDAR, S., BEATTIE, J. H., LODGE, J. K., JENAB, M., KEUN, H. & SCALBERT, A. 2011. Metabolomics and human nutrition. *Br J Nutr,* 105, 1277-83.

PSILANDER, N., FRANK, P., FLOCKHART, M. & SAHLIN, K. 2013. Exercise with low glycogen increases PGC-1alpha gene expression in human skeletal muscle. *Eur J Appl Physiol,* 113, 951-63.

PUIGSERVER, P., WU, Z., PARK, C. W., GRAVES, R., WRIGHT, M. & SPIEGELMAN, B. M. 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell,* 92, 829-39.

RAPOPORT, B. I. 2010. Metabolic factors limiting performance in marathon runners. *PLoS Comput Biol,* 6, e1000960.

RASANEN, M., NIINIKOSKI, H., KESKINEN, S., HELENIUS, H., TALVIA, S., RONNEMAA, T., VIIKARI, J. & SIMELL, O. 2003. Parental nutrition knowledge and nutrient intake in an atherosclerosis prevention project: the impact of child-targeted nutrition counselling. *Appetite,* 41, 69-77.

RASH, C. L., MALINAUSKAS, B. M., DUFFRIN, M. W., BARBER-HEIDAL, K. & OVERTON, R. F. 2008. Nutrition-related knowledge, attitude, and dietary intake of college track athletes. *The Sport Journal,* 11, 48-54.

RAVUSSIN, E., LILLIOJA, S., ANDERSON, T. E., CHRISTIN, L. & BOGARDUS, C. 1986. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest,* 78, 1568-78.

RAVUSSIN, E., LILLIOJA, S., KNOWLER, W. C., CHRISTIN, L., FREYMOND, D., ABBOTT, W. G., BOYCE, V., HOWARD, B. V. & BOGARDUS, C. 1988. Reduced rate of energy expenditure as a risk factor for body-weight gain. *N Engl J Med,* 318, 467-72.

RAYMOND-BARKER, P., PETROCZI, A. & QUESTED, E. 2007. Assessment of nutritional knowledge in female athletes susceptible to the Female Athlete Triad syndrome. *J Occup Med Toxicol,* 2, 10.

RICCIOTTI, E. & FITZGERALD, G. A. 2011. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol,* 31, 986-1000.

RODGERS, J. T., LERIN, C., GERHART-HINES, Z. & PUIGSERVER, P. 2008. Metabolic adaptations through the PGC-1a and SIRT1 pathways. *Federation of European Biochemical Societies,* 582, 46-53.

RODRIGUEZ, N. R. & GARLICK, P. J. 2008. Introduction to Protein Summit 2007: exploring the impact of high-quality protein on optimal health. *Am J Clin Nutr,* 87, 1551S-1553S.

ROLSTAD, S., ADLER, J. & RYDEN, A. 2011. Response burden and questionnaire length: is shorter better? A review and meta-analysis. *Value Health,* 14, 1101-8.

ROSENBLOOM, C. A., JONNALAGADDA, S. S. & SKINNER, R. 2002. Nutrition knowledge of collegiate athletes in a Division I National Collegiate Athletic Association institution. *Journal of the American Dietetic Association,* 102, 418-20.

ROWLANDS, D. S. & HOPKINS, W. G. 2002. Effects of high-fat and high-carbohydrate diets on metabolism and performance in cycling. *Metabolism,* 51, 678-90.

RUDERMAN, N. B., XU, X. J., NELSON, L., CACICEDO, J. M., SAHA, A. K., LAN, F. & IDO, Y. 2010. AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab,* 298, E751-60.

SAWYER-MORSE, M. K., SMOLIK, T., MOBLEY, C. & SAEGERT, M. 1989. Nutrition beliefs, practices, and perceptions of young dancers. *J Adolesc Health Care,* 10, 200-2.

SEARS, B. 1995. *The Zone Diet: A Dietary Road Map,* New York: , Regan Books.

SENEKAL, M. 2012. Genotype-based personalised nutrition for obesity prevention and treatment: are we there yet? *South African Journal of Clinical Nutrition,* 25, 9-14.

SHEPHERD, R. 1992. Nutrition Society--Nutrition and Behaviour Group. Symposium on Nutrition and Cognitive Efficiency, held at University of Reading, 3 April 1992. *Appetite,* 19, 157-8.

SIFRIM, D., DUPONT, L., BLONDEAU, K., ZHANG, X., TACK, J. & JANSSENS, J. 2005. Weakly acidic reflux in patients with chronic unexplained cough during 24 hour pressure, pH, and impedance monitoring. *Gut,* 54, 449-54.

SKOV, A. R., TOUBRO, S., RONN, B., HOLM, L. & ASTRUP, A. 1999. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord,* 23, 528-36.

SMITH-ROCKWELL, M., NICKOLS-RICHARDSON, S. M. & THYE, F. W. 2001. Nutrition knowledge, opinions, and practices of coaches and athletic trainers at a division 1 university. *Int J Sport Nutr Exerc Metab,* 11, 174-85.

SMITH, G. I., YOSHINO, J., KELLY, S. C., REEDS, D. N., OKUNADE, A., PATTERSON, B. W., KLEIN, S. & MITTENDORFER, B. 2016. High-Protein

Intake during Weight Loss Therapy Eliminates the Weight-Loss-Induced Improvement in Insulin Action in Obese Postmenopausal Women. *Cell Rep,* 17, 849-861.

SOLON-BIET, S. M., MITCHELL, S. J., COOGAN, S. C., COGGER, V. C., GOKARN, R., MCMAHON, A. C., RAUBENHEIMER, D., DE CABO, R., SIMPSON, S. J. & LE COUTEUR, D. G. 2015. Dietary Protein to Carbohydrate Ratio and Caloric Restriction: Comparing Metabolic Outcomes in Mice. *Cell Rep,* 11, 1529-34.

SPENDLOVE, J. K., HEANEY, S. E., GIFFORD, J. A., PRVAN, T., DENYER, G. S. & O'CONNOR, H. T. 2012. Evaluation of general nutrition knowledge in elite Australian athletes. *Br J Nutr,* 107, 1871-80.

STAFLEU, A., VAN STAVEREN, W. A., DE GRAAF, C., BUREMA, J. & HAUTVAST, J. G. 1996. Nutrition knowledge and attitudes towards high-fat foods and low-fat alternatives in three generations of women. *Eur J Clin Nutr,* 50, 33-41.

STEIN, T. P., RUMPLER, W. V., LESKIW, M. J., SCHLUTER, M. D., STAPLES, R. & BODWELL, C. E. 1991. Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man. *Metabolism,* 40, 478-83.

STELLINGWERFF, T., MAUGHAN, R. J. & BURKE, L. M. 2011. Nutrition for power sports: middle-distance running, track cycling, rowing, canoeing/kayaking, and swimming. *J Sports Sci,* 29 Suppl 1, S79-89.

STELLINGWERFF, T., SPRIET, L. L., WATT, M. J., KIMBER, N. E., HARGREAVES, M., HAWLEY, J. A. & BURKE, L. M. 2006. Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *Am J Physiol Endocrinol Metab,* 290, E380-8.

STIEGLER, P. & CUNLIFFE, A. 2006. The role of diet and exercise for the maintenance of fat-free mass and resting metabolic rate during weight loss. *Sports Med,* 36, 239-62.

STOCKLEY, L. 1985. Changes in habitual food intake during weighed inventory surveys and duplication diet collections. A short review. *Ecology of Food and Nutrition,* 17, 263-269.

STOEBER, J., OTTO, K., PESCHECK, E., BECKER, C. & STOLL, O. 2007. Perfectionism and competitive anxiety in athletes: Differentiating striving for perfection and negative reactions to imperfection. *Personality and Individual Differences,* 42, 959-969.

STUART, C. A., SHANGRAW, R. E., PETERS, E. J. & WOLFE, R. R. 1990. Effect of dietary protein on bed-rest-related changes in whole-body-protein synthesis. *Am J Clin Nutr,* 52, 509-14.

SU, S., KUO, C. & LIU, C. 2013. Diabetes and Mitochondria. *The Changhua Journal of Medicine,* 11, 1-7.

SUGIURA, K., SUZUKI, I. & KOBAYASHI, K. 1999. Nutritional intake of elite Japanese track-and-field athletes. *Int J Sport Nutr,* 9, 202-12.

SUWA, M., NAKANO, H., RADAK, Z. & KUMAGAI, S. 2008. Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor gamma coactivator-1alpha protein expressions in rat skeletal muscle. *Metabolism,* 57, 986-98.

THOMAS, D. T., ERDMAN, K. A. & BURKE, L. M. 2016. American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Med Sci Sports Exerc,* 48, 543-68.

THOMAS, S. E., KENDRICK, O. W. & EDDY, J. M. 1990. Modification of a Nutritional Questionnaire for Older Adults and the ability of its knowledge and attitude evaluations to predict dietary adequacy. *Journal of nutrition for the elderly,* 9, 35-63.

THOMPSON, F. E. & BYERS, T. 1994. Dietary assessment resource manual. *J Nutr,* 124, 2245S-2317S.

TIPTON, K. D., JEUKENDRUP, A. E., HESPEL, P. & INTERNATIONAL ASSOCIATION OF ATHLETICS, F. 2007. Nutrition for the sprinter. *J Sports Sci,* 25 Suppl 1, S5-15.

TRAPPE, S., LUDEN, N., MINCHEV, K., RAUE, U., JEMIOLO, B. & TRAPPE, T. A. 2015. Skeletal muscle signature of a champion sprint runner. *J Appl Physiol (1985),* 118, 1460-6.

VEGA, R. B., HUSS, J. M. & KELLY, D. P. 2000. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol,* 20, 1868-76.

VOGT, M., PUNTSCHART, A., HOWALD, H., MUELLER, B., MANNHART, C., GFELLER-TUESCHER, L., MULLIS, P. & HOPPELER, H. 2003. Effects of dietary fat on muscle substrates, metabolism, and performance in athletes. *Med Sci Sports Exerc,* 35, 952-60.

WALCOTT, W. L. & FAHEY, T. 2000. *The Metabolic Typing Diet*, Broadway Books, New York, United States of America.

WEINHEIMER, E. M., SANDS, L. P. & CAMPBELL, W. W. 2010. A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity. *Nutr Rev,* 68, 375-88.

WEINSIER, R. L., BOKER, J. R., MORGAN, S. L., FELDMAN, E. B., MOINUDDIN, J. F., MAMEL, J. J., DIGIROLAMO, M., BORUM, P. R., READ, M. S. & BROOKS, C. M. 1988. Cross-sectional study of nutrition knowledge and attitudes of medical students at three points in their medical training at 11 southeastern medical schools. *Am J Clin Nutr,* 48, 1-6.

WEINSIER, R. L., SCHUTZ, Y. & BRACCO, D. 1992. Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *Am J Clin Nutr,* 55, 790-4.

WEIR, J. B. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol,* 109, 1-9.

WHATI, L. H., SENEKAL, M., STEYN, N. P., NEL, J. H., LOMBARD, C. & NORRIS, S. 2005. Development of a reliable and valid nutritional knowledge questionnaire for urban South African adolescents. *Nutrition,* 21, 76-85.

WILLIAMS, R. J. 1956. *Biochemical individuality; the basis for the genetotrophic concept*.

WORLD-HEALTH-ORGANIZATION 2003. *Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation*, Diamond Pocket Books (P) Ltd.

WRIGHT, D. C., HAN, D.-H., GARCIA-ROVES, P. M., GEIGER, P. C., JONES, T. E. & HOLLOSZY, J. O. 2007. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1α expression. *Journal of Biological Chemistry,* 282, 194-199.

WU, Z., PUIGSERVER, P., ANDERSSON, U., ZHANG, C., ADELMANT, G., MOOTHA, V., TROY, A., CINTI, S., LOWELL, B., SCARPULLA, R. C. & SPIEGELMAN, B. M. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell,* 98, 115-24.

WYCHERLEY, T. P., MORAN, L. J., CLIFTON, P. M., NOAKES, M. & BRINKWORTH, G. D. 2012. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *Am J Clin Nutr,* 96, 1281-98.

ZINN, C., SCHOFIELD, G. & WALL, C. 2005. Development of a psychometrically valid and reliable sports nutrition knowledge questionnaire. *Journal of science and medicine in sport,* 8, 346-51.

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Appendix One

Metabolic typing questionnaire

1) Anger and Irritability
Sometimes we all get angry for good reason. But for some people, feelings of anger or irritability occur frequently. Skip this question if you do not experience anger or irritability that is affected by food.

- **a.** When I feel angry, eating meat or fatty food seems to make it worse.
- **b.** Sometimes eating relieves my anger and it does not really matter what I eat.
- **c.** I often notice that feelings of anger or irritability have abated after I eat something heavy and fatty like meat.

2) Anxiety
Do not answer this question if you do not experience anxiety that is influenced by food.

- **a.** When I feel anxious fruits or vegetables calm me down.
- **b.** Eating almost anything helps alleviate my anxiety.
- **c.** Heavy fatty food improves the way I feel and lessens my feelings of anxiety.

3) Ideal Breakfast
What kind of breakfast gives you the greatest energy, sense of well-being, peak performance, and satisfies your hunger the longest?

- **a.** Either no breakfast or something light like fruit and/or toast or cereal and/or milk or yogurt.
- **b.** egg(s), toast, fruit
- **c.** something heavy like eggs, bacon or sausage, hash browns, toast, or steak and eggs

4) Meal Preference
If you were to at a buffet dinner and were to cut loose and order anything without any guilt, what would the meal consist of?

- **a.** Lighter foods such as chicken, turkey, light fish, salads, vegetables, and I would sample various deserts.
- **b.** I would chose a combination of both a and c.
- **c.** Heavy rich, rich fatty foods: roast beef, beef stroganoff, pork chops, ribs, salmon, potatoes, gravy, few vegetables, or maybe a small salad with vinaigrette or blue cheese dressing; cheesecake or no desert.

5) Climate
Please select the choice that best describes how temperature affects you.

- **a.** I do best in warm or hot weather, I can't take the cold.
- **b.** Temperature does not matter that much. I do pretty well whether it is hot or cold.
- **c.** I do best in cool or cold temperatures. Can't take the heat.

6) Chest Pressure
Some people commonly experience chest pressure, a distinct sensation of pressure in the chest area. It often makes people feel as though a weight is on their chest, and tends to inhibit the ability to breathe. *(Only answer if you suffer from chest pressure)*

- **a.**
- **b.**
- **c.** I have a tendency to get or have problems with chest pressure.

7) Coffee
Please indicate how coffee affects you.

- **a.** I do well on coffee (as long as I do not drink to much)
- **b.** I can take it of leave it.
- **c.** I do not do well with coffee. It makes me jittery, jumpy, nervous, hyper, nauseated, shaky, or hungry.

8) Appetite at breakfast.
Appetites vary from day to day, but what is your overall tendency for your appetite at breakfast?

- **a.** Low, weak, or lacking.
- **b.** Normal. Don't notice it being weak or strong.
- **c.** Noticeably strong or above average.

9) Appetite at lunch.
Please highlight the answer that typically describes your appetite at lunch.

- **a.** Low, weak of lacking.
- **b.** Normal, Do not notice it being either strong or weak.
- **c.** Noticeably strong or above average.

10) Appetite at dinner.
Please highlight the answer that typically describes your appetite at dinner.

- **a.** Low, weak of lacking.
- **b.** Normal, Do not notice it being either strong or weak.
- **c.** Noticeably strong or above average.

11) Concentration
What foods worsen your ability to concentrate?

- **a.** Meat and/or fatty foods.

b. No particular kind of food seems to disrupt my concentration.
c. Fruit and vegetables and grain-based carbohydrates.

12) Coughing
Usually we think of coughing as something associated with illness. But some people naturally cough easily and often even when they are not sick. Typically the cough will be a dry cough and short in duration. If you are one of these people please highlight the answer.

a. I tend to cough every day

13) Cracking skin.
Some people have a problem with their skin cracking for no apparent reason. This typically occurs on the fingertips or on the feet, especially on the heal. If you are one of these people please highlight the answer.

a.
b.
c. I have a tendency to have problems with skin cracking.

14) Cravings
Most people have cravings for sugar when blood glucose is low; however do you have any craving for other foods?

a. Vegetables, fruits, grain-based products (bread, cereal, crackers)
b.
c. Salty, fatty foods (peanuts, cheese, crisps, meats..etc)

15) Dandruff
If you have a tendency to get dandruff please highlight the answer below

a.
b.
c. I tend to get dandruff.

16) Depression
Depression is often alleviated of worsened by what you eat. If you suffer from depression and have noticed a connection to food, select the appropriate choice below.

a. I seem to feel more depressed after eating meats and fatty foods (and less depressed after eating vegetables and fruits).
b.
c. I seem to feel more depressed after eating vegetables and fruit (and less depressed after eating meats and fatty foods).

17) Desserts
What is your general feeling towards having deserts after dinner?

- **a.** I really love sweets and/or I often need something sweet with a meal in order to feel satisfied.
- **b.** I enjoy desert from time to time, but can really take it or leave it.
- **c.** I do not really care for desserts the much; I may like something fatty or salty instead (like cheese, chips, popcorn) for a snack after a meal.

18) Dessert preferences
What are your favorite types of desserts? Which would you choose more often?

- **a.** Cakes, cookies and fruit pie.
- **b.** Truly no preference, I would choose different kinds each day.
- **c.** Heavier fatty types, like cheesecake creamy French pastries.

19) Ideal Dinner
What kind of meal works best for you at dinner time?

- **a.** Something light, like skinless chicken breast, rice, salad, maybe a little desert.
- **b.** Most foods work fine for me.
- **c.** I definitely do better with a heavier meal.

20) Ear colour
Please describe the response that best describes your ear colour.

- **a.** My ears tend to be pale, lighter than my skin tone.
- **b.** My ears tend to be the same shade as my face.
- **c.** My ears tend to be pink, red or darker than my face.

21) Eating before bed.
Eating before bed can help some people sleep, whilst is can clearly disrupt others sleep. How does eating anything before bed affect your sleep?

- **a.** It disrupts of worsens my sleep.
- **b.** It does not seem to make a difference, I can take it of leave it.
- **c.** It usually helps me sleep better.

22) Eating heavy foods before bed
What reaction do you have when eating heavy foods such as red meats, fowl, cheese and pastry before bed?

- **a.** It prevents or disturbs my sleep.
- **b.** It is usually ok, as long as I do not eat too much.
- **c.** It improves my sleep.

23) Eating light foods before bed.
What reaction do you have when eating light foods such as bread, toast, cereal, fruit or yogurt before bed?

a. I usually do not do well eating before sleep, but I definitely do better with lighter food.
b. I can take it or leave it.
c. It's better than nothing, but I do better with heavier food.

24) Eating sweets before bed
How do sweets affect your sleep?

a. Sweets do not interfere with me sleep at all.
b. Sweets sometimes bother my sleep.
c. I clearly do not do well eating sweets before sleep.

25) Eating frequently
How often do you need to eat in order to maximize your well-being and productivity?

a. 2 to 3 meals a day, and either no snacks, or a light snack.
b. 3 times a day, usually no snacks.
c. 3 meals or more a day and snacks, often something substantial.

26) Eating Habits
What is your attitude towards food?

a. I am unconcerned with food and eating; may forget to eat; rarely think about food; eat because I have to than because I want to.
b. I enjoy food, enjoy eating, rarely miss a meal, but do not really focus on food in any way.
c. I love food, love to eat, food is a big or central part of my life.

27) Eye moisture
Which of the following best describes your eyes?

a. My eyes tend to be dry.
b. I do no notice one way or the other.
c. My eyes tend to be very moist, even to the point of tearing.

28) Skipping meals
What happens to you when you go 4 hours or more without eating or skip a meal altogether?

a. Does not bother me, I can easily forget to eat.
b. I may not be at my best, but it does not really bother me.
c. I definitely feel worse, getting irritable, jittery, weak, tired, low on energy, depressed, or other negative symptoms.

29) Facial colouring
How would you characterize your facial colouring?

a. I am noticeably on the pale side.
b. I have average colouring.
c. I am noticeably darker (not from the sun), pink or flushed.

30) Facial complexion

How would you describe your facial complexion?

a. More dull and pasty.
b. Average.
c. Bright, radiant, clear.

31) Fatty foods
Value judgment aside, how much do you like or dislike fatty foods in general?

a. I do not really like fatty foods.
b. They are fine in moderation.
c. I love them and crave them and I would like them often if I knew they were good for me.

32) Fingernail thickness
How would you characterize the thickness of your fingernails?

a. My nails tend to be thick, strong and hard.
b. Seem average thickness.
c. I definitely seem to have thin and/or weak nails.

33) Fruit salad lunch
How would you feel after eating a (large) fruit salad with a little cottage cheese or yogurt for lunch?

a. It satisfies me; I do well on it and do not get hungry until dinner.
b. I do pretty well, but usually need a snack before dinner.
c. Pretty bed results. I usually get sleepy, tired, spacey, depressed anxious, irritable and/or hunger as a result and definitely need to eat something else before dinner.

34) Gaining weight
Which of the following bests describes your tendency to gain weight?

a. Meats and fatty foods cause me to gain weight.
b. No particular foods cause me to gain weight, but I will gain weight if I eat too much and do not get enough exercise.
c. I tend to gain weight eating too many carbohydrates (bread, pasta, other grains product, and/or fruit and vegetables.

35) Gag reflex
How would you best describe your gag reflex?

a. I rarely, if ever gag; it is hard to make me gag.
b. I probably have a normal gag reflex.
c. I easily gag and/or often gag.

36) Goose bumps
Are you prone to goose bumps?

a. I often get goose bumps.
b. I occasionally get goose bumps.

c. I rarely, if ever get goose bumps.

37) Energy boosters
What kind of foods generally boost your energy – and give you lasting energy?

a. Fruit, candy or pastry restores and gives me lasting energy.
b. Just about any food restores lasting food.
c. Meat and fatty food restores my energy and well – being.

38) Heavy-fat-meal reaction
Please choose the option the best describes your reaction to a high fat meal.

a. It decreases my well – being and energy, or makes me sleepy, or too full, or cause indigestion
b. Causes no special reaction in one way or another.
c. Increases my well – being; makes me feel good, energetic, satisfied, like 'I had a good meal'.

39) Hunger feelings
What kind of hunger feelings do you generally get from your body?

a. I rarely get hungry or feel real hunger, or have weak hunger feelings that pass quickly, or can easily go long periods without eating, or can forget about food altogether.
b. I have pretty normal hunger around meal times or when I am late for meals.
c. I often feel hungry, need to eat regularly and often, may get strong hunger sensations.

40) Energy drain
What kind of foods take your energy levels down a notch or two instead of giving you the boost you were looking for?

a. Meat and fatty foods generally makes me more tired, lowers my energy even more.
b. No foods in particular seems to take me down on a regular basis.
c. Fruit, pastry, or candy makes me worse, usually giving me a quick lift then a crash.

41) Insect bite or sting
How do insect bites or stings affect you?

a. Reactions tend to be mild and weak and tend to go away quickly.
b. Average reaction.
c. Clearly strong reaction, stronger than most, can involve above – average swelling, pain, itching, bruising, redness), and can take a long time to go away, even leaving discolouration after.

42) Insomnia
There are many types on insomnia. But with a certain type of insomnia, people routinely wake up in the middle of the night for reasons other than having to have to use the bathroom. Typically with this type of insomnia, people need to eat something

in order to fall back to sleep again. With this in mind, which one of the following applies to you?

a. I never/very rarely get this type of insomnia.
b. I occasionally wake up and need to eat to get back to sleep.
c. I often wake up and need to eat in order to go back to sleep. Eating something before going to sleep helps this problem or shortens the time that I am awake.

43) Itching eyes
From time to time everyone can experience itching eyes. This can happen when you have a cold, hay fever of allergies. But for many people itching eyes can be a common occurrence even when the above conditions are not present. This is the focus of the question. *(Only answer this question if it applies to you).*

a.
b.
c. I tend to get itching eyes often, even though I do not have a cold, allergy or hay fever.

44) Itching Skin
This question concerns itching skin that is not due to bites or stings. Everyone's skin itches from time to time. But some people find their skin itches regally on a daily basis, typically scalp, arms and calves. Because they are so used to it they may not even be conscious of the frequent scratching. *(Only answer this question if it applies to you).*

a.
b.
c. My skin tends to itch often.

45) Meal portions
When you eat out do you generally eat more than others, less than others, or about the same as others?

a. I do not eat that much, definitely less than the average.
b. I do not seem to eat more, or less than others.
c. I generally eat large portions of food, usually more than others.

46) Nose moisture
Please select the answer that best describes you when you are not ill or suffering from an allergic reaction.

a. My nose often seems too dry.
b. I do not notice my nose being too dry or too moist.
c. My nose often tends to run.

47) Fruit juice between meals
If you are hungry between meals, how does drinking a glass of fruit juice affect you?

a. It energizes me, satisfies me and works well until my next meal.
b. It is ok but it is not often the best snack for me.

c. Overall bad result. Can make me light headed, hungry after, jittery, shaky, nauseated, anxious, depressed etc.

48) Personality
Which of the following options best describes your natural tendency in social gatherings; or your preference with respect to day-to-day interactions with other people.

a. I seem to be more aloof, withdrawn or introverted.
b. I am pretty average, neither introverted nor extroverted.
c. I tend to be more social, a 'people person', or extroverted.

49) Potatoes
Whether you think potatoes are good for you or not, how do you feel about potatoes?

a. I do not really care for them that much or I do not really like them.
b. I can take them or leave them.
c. I love them; I could eat them almost every day.

50) Red meat
When you normally eat red meat how do you feel afterwards?

a. It decreases my energy and wellbeing. Can make me depressed and irritable.
b. I do not notice either one way or another.
c. I definitely feel good or better when I eat red meat.

51) Pupil size
Your pupils are the black centre portion or your eyes. The iris is the coloured section the surrounds the pupil. This question concerns the size of your pupil compares to the iris. Average means the pupil and the iris are approximately the same size. Larger means that the width of the pupil is clearly larger that the width of the iris. Smaller means that the width of the pupil is clearly smaller that the width of the iris. To answer first look in the mirror, but do so in an average lighted room, not too bright of too dark.

a. The size of my pupil seems to be larger than my iris
b. The size of my pupil seems to be the same size.
c. The size of my pupil seems to be smaller than my iris.

52) Salad for lunch.
If you ate a large vegetarian salad at lunch, what affect would it have on your productivity in the afternoon?

a. I do pretty well with that kind of lunch.
b. I can get by, but it is not the best type of lunch for me.
c. Bad results. Makes me feel either sleepy, tired, lethargic, hyper nervous of irritable.

53) Saliva quantity
Pleased select the option that most accurately characterizes your saliva production.

a. My mouth tends to be dry a lot of the time.

b. I do not notice that I have too little or too much saliva.
c. I tend to have a lot of saliva, or I have a tendency to drool.

54) Salty foods
Whether or not you feel that salt is good or bad for you, how do you feel about salt?

a. Foods often taste too salty, or I like my food only very slightly salted.
b. I do not really notice salt one way or another. Rarely seem to like too much or too little. Just use an average amount on foods.
c. I really love salt, or crave it. Like a lot of salt on foods, to the point that others think my food it too salty.

55) Snacking
Assume for the question that you eat 3 meals per day. If this is the case do you typically need to snack, or to eat something between meals? Or are those 3 meals all you need for peak performance?

a. I rarely if ever want or need snacks.
b. I occasionally want or need to snack between meals.
c. I often want or need to snack between meals.

56) Snack preference
Which of the following best describes your preference for snacks that you perform best on?

a. I generally do not need snacks, but if I do have one I usually prefer something sweet.
b. I generally want snacks and do pretty well on anything.
c. I definitely want and need snacks in order to do my best. Do poorly on sweets, but do well on protein and fat (meat, chicken, cheese, eggs and nuts).

57) Sneezing
We usually think of sneezing in connections with colds and allergies. But some people sneeze daily as a matter of course, even when they are not sick or plagued with allergies. For example some people sneeze routinely after eating. This question pertains to brief sneezing attacks, just one or two sneezes – not continuous, prolonged sneezing attacks. With this in mind, which best describes you?

a. I almost never sneeze, unless I have allergies.
b. I do sneeze from time to time when not sick or allergic, but not regularly.
c. I often regularly tend to sneeze and/or usually sneeze a little after eating.

58) Sociability
How would you describe you natural, innate tendency towards sociability, apart from the way your family and friends have influenced you in this?

- **a.** I tend to be a little antisocial, in that I enjoy being alone, feel awkward in social gathering or parties, and usually prefer to leave early or not go at all.
- **b.** I am in the middle, not really antisocial, but also not particularly compelled to be with others.
- **c.** I tend to be very social, a 'people person', and love company and to be with others, prefer not to be alone.

59) Sour foods
Which best describes your reaction to sour foods?

- **a.** I generally do not care for sour foods.
- **b.** I do not feel one way or the other. I do not like or dislike them more than any other food.
- **c.** I definitely like (some) sour foods or crave them.

60) Physical and mental stamina
Stamina refers to physical endurance, or the ability to work long hours without exhaustion. What type of foods best support your stamina?

- **a.** My stamina is better when I eat lighter foods like chicken, fish, fruit, vegetables or grains.
- **b.** My stamina is better when I eat pretty much any wholesome food.
- **c.** My stamina is better when I eat heavy foods or fatty foods.

61) Consuming sweets
How do you react when you eat sweets all by themselves?

- **a.** Sweets do not bother me even if I eat them by themselves. Generally sweets satisfy my appetite and do not produce bad reactions.
- **b.** I am sometimes bothered when eating sweets by themselves, and often they do not satisfy my appetite.
- **c.** I usually do not do well eating sweets by themselves. They usually produce some manner of a bad reaction and/or create a desire for more sweets.

62) Meat for breakfast
How do you feel after eating meat for breakfast, as opposed to going without it?

- **a.** I do not feel as well as I do without it. Tends to make me sleepier, lethargic, angry, irritable, thirsty, or causes me to lose my energy by midmorning.
- **b.** I can take it or leave it, it varies.
- **c.** I feel much better with it: more energetic, have good stamina, keeps me going without getting hungry before lunch.

63) Red meat for lunch
How do you feel when eating red meat for lunch, as opposed to going without it?

- **a.** I do not feel as well as I do without it. Tends to make me sleepier, lethargic, angry, irritable, thirsty, or causes me to lose my energy by mid-afternoon.

b. I can take it or leave it, it varies.
c. I feel much better with it: more energetic, have good stamina, keeps me going without getting hungry before dinner.

64) Red meat for dinner
How do you feel when eating red meat for dinner, as opposed to going without it?

a. I do not feel as well as I do without it. Tends to make me sleepier, lethargic,
b. angry, irritable, thirsty, or causes me to lose my energy.
c. I can take it or leave it, it varies.
d. I feel much better with it: more energetic, have good stamina, keeps me going without getting hungry before bedtime

65) Dinner preference.
If you had a long night ahead of you, which dinner would you choose to give you the best stamina alertness and energy?

a. Dinner plate 1 – Skinless chicken breast, rice salad and apple pie.
b. Dinner plate 2 – Combination plate, including a little bit from both plates 1 and 3.
c. Dinner plate 3 – Pot roast cooked with carrots, onions and potatoes, served with biscuits and gravy. Cheesecake.

Appendix Two

Metabolic Typing Diet Examples

Eating Guidelines for Diet Plan 1-O - Slow Oxidizer
Recommended by Healthexcel

Bold = Ideal (eat ideal foods at every meal)
Black = Neutral (ok, but emphasize "Ideal" foods)
Italics = Caution (eat rarely or only for variety)
~~Strikethrough~~ = **Avoid** (don't eat these foods)

Meats	Poultry	Seafood		
Pork (ham, chops)	Chicken (white meat)	Bass (freshwater)	Whitefish	*Trout*
Beef	Quail	Bass (sea)	*Abalone*	*Tuna*
Lamb	Turkey (white meat)	Catfish	*Clams*	~~Anchovy~~
Pork (bacon)	Chicken (dark meat)	Cod	*Crab*	~~Caviar~~
Rabbit	Cornish Hen	Grouper	*Crayfish*	~~Herring~~
~~Buffalo~~	Turkey (dark meat)	Halibut	*Lobster*	~~Mackerel~~
~~Elk~~	*Duck*	Mahi-mahi	*Octopus*	~~Mussels~~
~~Heart (beef)~~	*Goose*	Perch	*Oysters*	~~Sardine~~
~~Kidney (beef)~~	*Pheasant*	Rockfish	*Pompano*	~~Scallop~~
~~Liver (beef)~~		Roughy	*Salmon*	~~Shrimp~~
~~Venison~~		Snapper	*Shark*	
		Swordfish	*Squid*	

Legumes		Beverages	
Garbanzo Beans	Lentils	Almond Milk	*Fruit Juices*
Green Beans	Lima Beans	Oat Milk	*Water (carbonated)*
Navy Beans	Mung Beans	Rice Milk	*Wine (red)*
Pink Beans	Red Beans	Tea (black)	*Wine (white)*
Pinto Beans	~~Soy Beans~~	Tea (green)	~~Liquor~~
White Beans	~~Tofu~~	Tea (herbal)	~~Soft Drinks (colas)~~
Aduki Beans		Vegetable Juices	~~Soy Milk~~
Black Beans		Water (distilled)	~~Water (tap)~~
Black-eyed Peas		Water (pure, bottled)	
Fava Beans		Coffee (caffeinated)	
Great Northern Beans		Coffee (decaf)	
Green Peas		*Beer*	

Dairy and Eggs		Nuts and Seeds	Grains	Greens
Blue Cheese	Ricotta	Almonds	Rice (basmati)	Beet Greens
Brie	Roquefort	Cashews	Rice (brown)	Cilantro
Camembert	Whey	Chestnuts	Spelt	Endive
Cheddar	Yogurt	Pine Nuts	Triticale	Kale
Colby	Buttermilk	Poppy Seeds	Wheat	Lettuce (bibb)
Cottage Cheese (lite)	Cottage Cheese	Sesame Seeds	Wild Rice	Lettuce (iceberg)
Edam	Cream (half and half)	Sunflower Seeds	Amaranth	Lettuce (loose-leaf)
Eggs, Chicken (whites)	Eggs, Duck (whole)	*Brazil Nuts*	Barley	Lettuce (romaine)
Eggs, Chicken (yolks)	Goats Milk	*Filberts*	Buckwheat	Mustard Greens
Feta	Gruyere	*Hickory Nuts*	Kamut	Radicchio
Goat Cheese	Milk (whole)	*Macadamia Nuts*	Millet	Swiss Chard
Gouda	Parmesan	*Peanuts*	Oat	Watercress
Milk (2%)	Romano	*Pecans*	Quinoa	Arugula
Milk (skim)	Swiss	*Pistachios*	Rye	Collard Greens
Monterey Jack	*Cream Cheese*	*Pumpkin Seeds*	~~Rice (plain, white)~~	Dandelion Greens
Mozzarella	~~Ice Cream~~	*Walnuts*		Sprouts (alfalfa)
Muenster	~~Sherbet~~			Sprouts (bean)
Neufchatel	~~Sour Cream~~			Turnip Greens
Provolone				*Spinach*

Vegetables			Sea Vegetables
Bok Choy	Bamboo Shoots	*Avocado*	Dulse
Broccoli	Beet	*Cauliflower*	Irish Moss
Brussels Sprout	Carrot	*Mushroom (all varieties)*	Kelp
Cabbage	Celery	*Olive (all varieties)*	*Agar*
Cucumber	Corn		*Laver*
Daikon	Eggplant		*Wakame*
Garlic	Fennel		
Ginger Root	Jerusalem Artichoke		
Kohlrabi	Jicama		
Leek	Parsnip		
Okra	Potato (all varieties)		
Onion	Pumpkin		
Pepper (bell, all colors)	Rutabaga		
Pepper (hot, all colors)	Squash (summer)		
Radish	Squash (winter)		
Shallot	Turnip		
Sweet Potato (yam)	Water Chestnuts		
Tomato	*Artichoke*		
Zucchini	*Asparagus*		

Fruits			Oils and Fats	
Apricots	Honeydew Melon	Rhubarb	Butter (unsalted)	Sunflower Oil
Blackberries	Kiwifruit	Strawberries	Coconut Oil	Wheat Germ Oil
Blueberries	Kumquat	Watermelon	Flax Oil	~~Canola Oil~~
Boysenberries	Loganberries	Apples	Ghee (clarified butter)	~~Corn Oil~~
Cantaloupe	Mango	Banana	Olive Oil	~~Cottonseed Oil~~
Casaba Melon	Nectarines	Coconut	Almond Oil	~~Margarine~~
Cherries	Papaya	Grapefruit	Black Currant Oil	~~Palm Kernel Oil~~
Cranberries	Peaches	Lemons	Borage Oil	
Currants	Persimmon	Limes	Butter (salted)	
Dates	Pineapple	Oranges	Evening Primrose Oil	
Elderberries	Plums	Pears	Fish Oil	
Figs	Pomegranate	Tangerines	Hemp Oil	
Gooseberries	Prunes		Peanut Oil	
Grapes	Raisins		Safflower Oil	
Guava	Raspberries		Sesame Oil	

Herbs spices and Seasonings			
Basil	Carob	Mustard	Vinegar (wine)
Chive	Cayenne	Mustard Seed	Wasabi
Dill Weed	Chervil	Nutmeg	*Chocolate*
Ginger	Chili Powder	Paprika	*Mayonnaise*
Marjoram	Cinnamon	Pepper (ground black)	*Molasses*
Oregano	Cloves	Peppermint	*Sugar (brown,unrefined)*
Parsley	Coriander	Saffron	*Vanilla (extract)*
Rosemary	Cumin	Sage	~~Artificial Sweeteners~~
Tarragon	Curry Power	Salt (sea salt,unrefined)	~~Ketchup~~
Thyme	Fennel Seed	Savory	~~Salt (iodized)~~
Turmeric	Fenugreek	Soy Sauce	~~Salt (low sodium)~~
Anise	Garlic Powder	Spearmint	~~Sugar (brown)~~
Bay Leaf	Honey	Vinegar (apple cider)	~~Sugar (white)~~
Caraway	Horseradish	Vinegar (balsamic)	
Cardamom	Mace	Vinegar (rice)	

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These Eating Guidelines may require modification as your health status changes.
Schedule a follow-up with your practitioner.

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Eating Guidelines for Diet Plan 2-O - Fast Oxidizer
Recommended by Healthexcel

Bold = Ideal (eat ideal foods at every meal)
Black = Neutral (ok, but emphasize "Ideal" foods)
Italics = Caution (eat rarely or only for variety)
~~Strikethrough~~ = **Avoid** (don't eat these foods)

Meats	Poultry	Seafood			Legumes		Beverages	
Beef	**Chicken (dark meat)**	**Anchovy**	**Crayfish**	*Cod*	**Black Beans**	*Pink Beans*	**Water (distilled)**	*Wine (white)*
Buffalo	**Cornish Hen**	**Caviar**	**Lobster**	*Grouper*	**Great Northern Beans**	*Pinto Beans*	**Water (pure, bottled)**	~~Coffee (decaf)~~
Elk	**Duck**	**Herring**	**Octopus**	*Halibut*	**Green Peas**	*White Beans*	*Almond Milk*	~~Fruit Juices~~
Heart (beef)	**Goose**	**Mackerel**	**Oysters**	*Mahi-mahi*	**Lentils**	~~Black-eyed Peas~~	*Beer*	~~Liquor~~
Kidney (beef)	**Pheasant**	**Mussels**	**Pompano**	*Perch*	**Adzuki Beans**	~~Soy Beans~~	*Coffee (caffeinated)*	~~Soft Drinks (colas)~~
Liver (beef)	**Turkey (dark meat)**	**Salmon**	**Shark**	*Rockfish*	**Fava Beans**	~~Tofu~~	*Oat Milk*	~~Soy Milk~~
Venison	**Chicken (white meat)**	**Sardine**	**Squid**	*Roughy*	**Green Beans**		*Rice Milk*	~~Water (carbonated)~~
Lamb	**Quail**	**Scallop**	**Trout**	*Snapper*	**Lima Beans**		*Tea (black)*	~~Water (tap)~~
Pork (bacon)	**Turkey (white meat)**	**Shrimp**	**Tuna**	*Swordfish*	**Mung Beans**		*Tea (green)*	
Pork (ham, chops)		**Abalone**	*Bass (freshwater)*	*Whitefish*	**Red Beans**		*Tea (herbal)*	
Rabbit		**Clams**	*Bass (sea)*		*Garbanzo Beans*		*Vegetable Juices*	
		Crab	*Catfish*		*Navy Beans*		*Wine (red)*	

Dairy and Eggs		Nuts and Seeds	Grains	Greens	Vegetables			Sea Vegetables
Buttermilk	**Colby**	**Poppy Seeds**	**Amaranth**	**Spinach**	**Artichoke**	*Jicama*	~~Potato (all varieties)~~	**Agar**
Cottage Cheese	**Edam**	**Pumpkin Seeds**	**Spelt**	**Sprouts (alfalfa)**	**Asparagus**	*Kohlrabi*	~~Pumpkin~~	**Laver**
Cream (half and half)	**Feta**	**Sesame Seeds**	*Kamut*	**Sprouts (bean)**	**Avocado**	*Okra*	~~Rutabaga~~	**Wakame**
Cream Cheese	**Gouda**	**Sunflower Seeds**	*Oat*	*Arugula*	**Cauliflower**	*Radish*	~~Sweet Potato (yam)~~	**Dulse**
Eggs, Chicken (whites)	**Monterey Jack**	**Almonds**	*Quinoa*	*Cilantro*	**Celery**	*Shallot*		**Irish Moss**
Eggs, Chicken (yolks)	**Mozzarella**	**Brazil Nuts**	*Triticale*	*Collard Greens*	**Mushroom (all varieties)**	*Squash (summer)*		**Kelp**
Eggs, Duck (whole)	**Muenster**	**Cashews**	~~Barley~~	*Dandelion Greens*	**Olive (all varieties)**	*Squash (winter)*		
Goat Cheese	**Neufchatel**	**Chestnuts**	~~Buckwheat~~	*Kale*	**Carrot**	*Tomato*		
Goats Milk	**Provolone**	**Filberts**	~~Millet~~	*Lettuce (bibb)*	**Corn**	*Turnip*		
Gruyere	**Ricotta**	**Hickory Nuts**	~~Rice (basmati)~~	*Lettuce (iceberg)*	*Bamboo Shoots*	*Water Chestnuts*		
Milk (whole)	**Roquefort**	**Macadamia Nuts**	~~Rice (brown)~~	*Lettuce (loose-leaf)*	*Bok Choy*	*Zucchini*		
Parmesan	**Whey**	**Peanuts**	~~Rice (plain, white)~~	*Lettuce (romaine)*	*Cabbage*	~~Beet~~		
Romano	**Yogurt**	**Pecans**	~~Rye~~	*Radicchio*	*Cucumber*	~~Broccoli~~		
Sour Cream	*Cottage Cheese (lite)*	**Pine Nuts**	~~Wheat~~	*Turnip Greens*	*Daikon*	~~Brussels Sprout~~		
Swiss	*Milk (2%)*	**Pistachios**	~~Wild Rice~~	*Watercress*	*Eggplant*	~~Leek~~		
Blue Cheese	*Milk (skim)*	**Walnuts**		~~Beet Greens~~	*Fennel*	~~Onion~~		
Brie	~~Ice Cream~~			~~Endive~~	*Garlic*	~~Parsnip~~		
Camembert	~~Sherbet~~			~~Mustard Greens~~	*Ginger Root*	~~Pepper (bell, all colors)~~		
Cheddar				~~Swiss Chard~~	*Jerusalem Artichoke*	~~Pepper (hot, all colors)~~		

Fruits			Oils and Fats		Herbs spices and Seasonings			
Apples	*Guava*	~~Figs~~	**Butter (salted)**	**Sunflower Oil**	**Salt (sea salt,unrefined)**	**Curry Power**	**Rosemary**	*Vinegar (rice)*
Banana	*Kiwifruit*	~~Grapefruit~~	**Butter (unsalted)**	**Wheat Germ Oil**	**Soy Sauce**	**Dill Weed**	**Saffron**	*Vinegar (wine)*
Coconut	*Kumquat*	~~Honeydew Melon~~	**Coconut Oil**	~~Canola Oil~~	**Anise**	**Fennel Seed**	**Sage**	~~Artificial Sweeteners~~
Pears	*Loganberries*	~~Lemons~~	**Fish Oil**	~~Corn Oil~~	**Basil**	**Fenugreek**	**Savory**	~~Chocolate~~
Cherries	*Mango*	~~Limes~~	**Flax Oil**	~~Cottonseed Oil~~	**Bay Leaf**	**Garlic Powder**	**Spearmint**	~~Horseradish~~
Elderberries	*Papaya*	~~Nectarines~~	**Ghee (clarified butter)**	~~Margarine~~	**Caraway**	**Ginger**	**Tarragon**	~~Ketchup~~
Peaches	*Plums*	~~Oranges~~	**Olive Oil**	~~Palm Kernel Oil~~	**Cardamom**	**Honey**	**Thyme**	~~Molasses~~
Pomegranate	*Raspberries*	~~Persimmon~~	**Almond Oil**		**Carob**	**Mace**	**Turmeric**	~~Mustard~~
Blackberries	*Rhubarb*	~~Pineapple~~	**Black Currant Oil**		**Cayenne**	**Marjoram**	**Vanilla (extract)**	~~Salt (iodized)~~
Blueberries	*Strawberries*	~~Prunes~~	**Borage Oil**		**Chervil**	**Mayonnaise**	**Wasabi**	~~Salt (low sodium)~~
Boysenberries	*Watermelon*	~~Raisins~~	**Evening Primrose Oil**		**Chili Powder**	**Mustard Seed**	*Chive*	~~Sugar (brown)~~
Cantaloupe	~~Apricots~~	~~Tangerines~~	**Hemp Oil**		**Cinnamon**	**Nutmeg**	*Parsley*	~~Sugar (brown,unrefined)~~
Cranberries	~~Casaba Melon~~		**Peanut Oil**		**Cloves**	**Oregano**	*Pepper (ground black)*	~~Sugar (white)~~
Gooseberries	~~Currants~~		**Safflower Oil**		**Coriander**	**Paprika**	*Vinegar (apple cider)*	
Grapes	~~Dates~~		**Sesame Oil**		**Cumin**	**Peppermint**	*Vinegar (balsamic)*	

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Schedule a follow-up with your practitioner.

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Eating Guidelines for Diet Plan 3-O - Mixed Oxidizer
Mixed 1 (slow oxidation support)
Recommended by Healthexcel

Bold = Ideal (eat ideal foods at every meal)
Black = Neutral (ok, but emphasize "Ideal" foods)
Italics = Caution (eat rarely or only for variety)
~~Strikethrough~~ = **Avoid** (don't eat these foods)

Meats	Poultry	Seafood			Legumes		Beverages	
Pork (ham, chops)	**Chicken (white meat)**	**Bass (freshwater)**	**Whitefish**	*Trout*	**Garbanzo Beans**	**Lentils**	**Almond Milk**	*Fruit Juices*
Beef	**Quail**	**Bass (sea)**	*Abalone*	*Tuna*	**Green Beans**	**Lima Beans**	**Oat Milk**	*Water (carbonated)*
Lamb	**Turkey (white meat)**	**Catfish**	*Clams*	~~Anchovy~~	**Navy Beans**	**Mung Beans**	**Rice Milk**	*Wine (red)*
Pork (bacon)	**Chicken (dark meat)**	**Cod**	*Crab*	~~Caviar~~	**Pink Beans**	**Red Beans**	**Tea (black)**	*Wine (white)*
Rabbit	**Cornish Hen**	**Grouper**	*Crayfish*	~~Herring~~	**Pinto Beans**	~~Soy Beans~~	**Tea (green)**	~~Liquor~~
~~Buffalo~~	**Turkey (dark meat)**	**Halibut**	*Lobster*	~~Mackerel~~	**White Beans**	~~Tofu~~	**Tea (herbal)**	~~Soft Drinks (colas)~~
~~Elk~~	*Duck*	**Mahi-mahi**	*Octopus*	~~Mussels~~	**Aduki Beans**		**Vegetable Juices**	~~Soy Milk~~
~~Heart (beef)~~	*Goose*	**Perch**	*Oysters*	~~Sardine~~	**Black Beans**		**Water (distilled)**	~~Water (tap)~~
~~Kidney (beef)~~	*Pheasant*	**Rockfish**	*Pompano*	~~Scallop~~	**Black-eyed Peas**		**Water (pure, bottled)**	
~~Liver (beef)~~		**Roughy**	*Salmon*	~~Shrimp~~	**Fava Beans**		**Coffee (caffeinated)**	
~~Venison~~		**Snapper**	*Shark*		**Great Northern Beans**		**Coffee (decaf)**	
		Swordfish	*Squid*		**Green Peas**		*Beer*	

Dairy and Eggs		Nuts and Seeds	Grains	Greens	Vegetables			Sea Vegetables
Blue Cheese	**Ricotta**	**Almonds**	**Rice (basmati)**	**Beet Greens**	**Bok Choy**	**Bamboo Shoots**	*Avocado*	**Dulse**
Brie	**Roquefort**	**Cashews**	**Rice (brown)**	**Cilantro**	**Broccoli**	**Beet**	*Cauliflower*	**Irish Moss**
Camembert	**Whey**	**Chestnuts**	**Spelt**	**Endive**	**Brussels Sprout**	**Carrot**	*Mushroom (all varieties)*	**Kelp**
Cheddar	**Yogurt**	**Pine Nuts**	**Triticale**	**Kale**	**Cabbage**	**Celery**	*Olive (all varieties)*	*Agar*
Colby	**Buttermilk**	**Poppy Seeds**	**Wheat**	**Lettuce (bibb)**	**Cucumber**	**Corn**		*Laver*
Cottage Cheese (lite)	**Cottage Cheese**	**Sesame Seeds**	**Wild Rice**	**Lettuce (iceberg)**	**Daikon**	**Eggplant**		*Wakame*
Edam	**Cream (half and half)**	**Sunflower Seeds**	**Amaranth**	**Lettuce (loose-leaf)**	**Garlic**	**Fennel**		
Eggs, Chicken (whites)	**Eggs, Duck (whole)**	*Brazil Nuts*	**Barley**	**Lettuce (romaine)**	**Ginger Root**	**Jerusalem Artichoke**		
Eggs, Chicken (yolks)	**Goats Milk**	*Filberts*	**Buckwheat**	**Mustard Greens**	**Kohlrabi**	**Jicama**		
Feta	**Gruyere**	*Hickory Nuts*	**Kamut**	**Radicchio**	**Leek**	**Parsnip**		
Goat Cheese	**Milk (whole)**	*Macadamia Nuts*	**Millet**	**Swiss Chard**	**Okra**	**Potato (all varieties)**		
Gouda	**Parmesan**	*Peanuts*	**Oat**	**Watercress**	**Onion**	**Pumpkin**		
Milk (2%)	**Romano**	*Pecans*	**Quinoa**	**Arugula**	**Pepper (bell, all colors)**	**Rutabaga**		
Milk (skim)	**Swiss**	*Pistachios*	**Rye**	**Collard Greens**	**Pepper (hot, all colors)**	**Squash (summer)**		
Monterey Jack	*Cream Cheese*	*Pumpkin Seeds*	~~Rice (plain, white)~~	**Dandelion Greens**	**Radish**	**Squash (winter)**		
Mozzarella	~~Ice Cream~~	*Walnuts*		**Sprouts (alfalfa)**	**Shallot**	**Turnip**		
Muenster	~~Sherbet~~			**Sprouts (bean)**	**Sweet Potato (yam)**	**Water Chestnuts**		
Neufchatel	~~Sour Cream~~			**Turnip Greens**	**Tomato**	*Artichoke*		
Provolone				*Spinach*	**Zucchini**	*Asparagus*		

Fruits			Oils and Fats		Herbs spices and Seasonings			
Apricots	**Honeydew Melon**	**Rhubarb**	**Butter (unsalted)**	**Sunflower Oil**	**Basil**	**Carob**	**Mustard**	**Vinegar (wine)**
Blackberries	**Kiwifruit**	**Strawberries**	**Coconut Oil**	**Wheat Germ Oil**	**Chive**	**Cayenne**	**Mustard Seed**	**Wasabi**
Blueberries	**Kumquat**	**Watermelon**	**Flax Oil**	~~Canola Oil~~	**Dill Weed**	**Chervil**	**Nutmeg**	*Chocolate*
Boysenberries	**Loganberries**	**Apples**	**Ghee (clarified butter)**	~~Corn Oil~~	**Ginger**	**Chili Powder**	**Paprika**	*Mayonnaise*
Cantaloupe	**Mango**	**Banana**	**Olive Oil**	~~Cottonseed Oil~~	**Marjoram**	**Cinnamon**	**Pepper (ground black)**	*Molasses*
Casaba Melon	**Nectarines**	**Coconut**	**Almond Oil**	~~Margarine~~	**Oregano**	**Cloves**	**Peppermint**	*Sugar (brown,unrefined)*
Cherries	**Papaya**	**Grapefruit**	**Black Currant Oil**	~~Palm Kernel Oil~~	**Parsley**	**Coriander**	**Saffron**	*Vanilla (extract)*
Cranberries	**Peaches**	**Lemons**	**Borage Oil**		**Rosemary**	**Cumin**	**Sage**	~~Artificial Sweeteners~~
Currants	**Persimmon**	**Limes**	**Butter (salted)**		**Tarragon**	**Curry Power**	**Salt (sea salt,unrefined)**	~~Ketchup~~
Dates	**Pineapple**	**Oranges**	**Evening Primrose Oil**		**Thyme**	**Fennel Seed**	**Savory**	~~Salt (iodized)~~
Elderberries	**Plums**	**Pears**	**Fish Oil**		**Turmeric**	**Fenugreek**	**Soy Sauce**	~~Salt (low sodium)~~
Figs	**Pomegranate**	**Tangerines**	**Hemp Oil**		**Anise**	**Garlic Powder**	**Spearmint**	~~Sugar (brown)~~
Gooseberries	**Prunes**		**Peanut Oil**		**Bay Leaf**	**Honey**	**Vinegar (apple cider)**	~~Sugar (white)~~
Grapes	**Raisins**		**Safflower Oil**		**Caraway**	**Horseradish**	**Vinegar (balsamic)**	
Guava	**Raspberries**		**Sesame Oil**		**Cardamom**	**Mace**	**Vinegar (rice)**	

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Schedule a follow-up with your practitioner.

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Appendix Three

Micro Muscle Biopsy SOP

PURPOSE:

To describe the surgical procedure for the removal of muscle tissue from human participants for the purpose of biochemical, histochemical and molecular analysis.

SCOPE:

This procedure is applied to the persons performing the procedure and those involved in assisting.

RESPONSIBILITIES:

- The lead researcher (researcher who takes the biopsy) is fully responsible for the procedure and the appropriate experience of those assisting in the procedure.
- All those participating in the procedure MUST read the appropriate RA's and SOP.

REFERENCES:

- NHS good hand washing techniques
- Protocol of safe working 1.4
- Human Tissue Authority, Code of Practice 9: Research
- Automatic Biopsy Instrument instructions
- Lidocaine information sheet
- Current risk assessment for muscle biopsy

EQUIPMENT AND MATERIALS:

- Equipment
    - Biopsy instrument
    - Micro biopsy needle and cannula
    - Surgical trolley
    - Liquid nitrogen – Stored in Dewar
    - Blunt edged forceps (tweezers)
    - Steel sample well
    - Sharps bin

    - Cryo-gloves
- Materials
    - Biopsy couch
    - Blue r oll
    - Hydrex surgical hand scrub
    - Betadine surgical scrub for skin antiseptic
    - Sterile drape
    - Gauze
    - Latex free gloves
    - Disposable plastic apron
    - 2.5 ml syringe with 25G needle
    - Sterile surgical scalpel blade (No. 11)
    - Plasters
    - Cryovials (5 ml)
    - Ice
    - -80 ^{o}C freezer
- Chemicals
    - Lidocaine hydrochloride (1% w/v)

PROCEDURE

- ONLY carry out the procedure if the participant has been informed of the full procedure, signed all relevant documents and provided informed consent.
- It should be ensured that the participant has had no previous complications when having blood or biopsy taken.
- It should be ensured that the participant has no known allergies.
- Check all equipment is in full working order, and the room has adequate lighting.
- All procedures should be preformed in aseptic manner with aid from an assistant (i.e. the assistant opens all packaging without touching the equipment).
- Lay out all equipment needed for the procedure in a neat/easy to access order.
- Wash hands thoroughly with Hydrex surgical hand scrub, following the SOP for good hand washing technique.
- Open the sterile drape and place it on the stainless steel trolley.
- Expose the target thigh; ensuring all clothing is rolled up/removed to avoid any contact with the biopsy area.
- Pour a sufficient amount of Betadine onto a double thickness piece of gauze.
- Prepare the biopsy site by rubbing the Betadine over an area sufficient to ensure the biopsy site is sterile and can be touched and manipulated (cover and area approximately 15 x 15 cm).

- Place the gauze on the thigh on an area that is covered in Betadine for use later.
- Ask the participant to contract their quadriceps to help define an appropriate biopsy site on the Vastus Lateralis muscle.
- Using a water soluble pen, draw a circle around the area isolated for the biopsy site (approximately 2 - 3 cm diameter). Re-rub the biopsy site with the Betadine ensuring the pen mark is covered.
- Open the 2.5ml syringe and needle package along with the ampule of lidocaine hydrochloride (1 % w/v).
- Draw up approximately 2.5 ml of lidocaine hydrochloride (1% w/v) into the syringe. Expel any air bubbles present in the syringe.
- Inject the Lidocaine hydrochloride under the surface of the skin in multiple directions. Take care during the procedure to ensure it is not being injected into a small blood vessel.
- Allow a minimum of 5 minutes for the anesthetic to take effect. (If during this time any sign of allergic reaction is seen, follow the procedure outlined in 7.5.2)
- Whilst waiting for the anesthetic to take effect open up the sterile semi-automated biopsy instrument pack and cannula and place on the sterile drape. Loosen all sheath and components.
- Once anesthetic has taken effect, insert the cannula through the skin, sub-cutaneous fat and fascia into the Vastus Lateralis. (A significant amount of pressure is needed to pierce the fascia).
- Prime (as per manufacture guidelines) the biopsy instrument.
- Insert the primed biopsy instrument all the way into the cannula and activate (sampling the muscle tissue)
- Remove the instrument immediately from the cannula.
- Whilst being held by the investigator the sheath of the needle is opened to expose the tissue. With sterile scalpel blade the tissue sample is removed from the needle (ensuring the biopsy instrument is not touched by a non-sterile object – this allows for repeat samples).
- If further samples are required, up to 4 passed can occur through the cannular on the same occasion using the same needle.
- The assistant freezes the tissue appropriately and places sample into storage according to the relevant SOP (See 7.0 – 7.7).
- Remove the cannula (and place in the sharps bin), using the gauze with Betadine on apply pressure on the biopsy site (for a minimum of 5 minutes)
- Once the bleeding has stopped place a plaster over the biopsy site.
- Instruct the participant on all aftercare and how to change the dressing.
- The plaster is kept on for 24 hours and must be kept dry.
- All contaminated consumables are disposed of using the correct SOP for disposure of clinical waste.
- The investigator and assistant must wash hands appropriately.

- The biopsy instrument is cleaned and steralised according to the manufactures guidelines (see manufactures guidelines) (or disposed of if single use)

TISSUE STORAGE AND RENDERING ACELLULAR

- Place the steel sample bowl on to the polystyrene base.
- Using Cryo-gloves pour approximately 100 ml of liquid nitrogen in to a steel bowl.
- Using a sterile blade (Swann Morton No.11) scrape the muscle tissue from the biopsy needle.
- IT IS IMPERATIVE THAT NOTHING OTHER THAN THE STERILE BLADE TOUCHES THE NEEDLE IF REPEAT SAMPLES ARE TO BE TAKEN.
- Place the muscle tissue into the liquid nitrogen; leave until the liquid nitrogen has evaporated. (If the tissue is not fully frozen, pour another 50 mls of liquid nitrogen into the metal beaker).
- Once frozen, using clean blunt forceps place the tissue into a cryovial, label and store in a -80 freezer with the intent to render acellular.
- From the time the sample is taken a maximum of 7-days storage is allowed.
- The acellular rendering of the sample within 7-days complies with the Human Tissue Authority (HTA) guidelines (Licensing for research code 60: http://www.hta.gov.uk/legislationpoliciesandcodesofpractice/codesofpractice/code9research.cfm?FaArea1=customwidgets.content_view_1&cit_id=764&cit_parent_cit_id=757) (Also HTA licensing flow chart).
- Extract the DNA/RNA within 7 days via the proposed method the render the tissue acellular.

SPECIAL NOTES: HEALTH & SAFETY

- Read appropriate risk assessment for this procedure
    - Summary of risk for experimenter
- Low temperature hazard
- Sharps hazard
- Biohazard
- Key safety issues (to minimize above risk)
    - Low temperature hazard: ALWAYS wear a long sleeved lab coat, thermally insulated gloves and suitable footwear and safety glasses when handling liquid nitrogen.
    - Sharps hazard: Handle all needles and surgical blades with care and dispose of appropriately and immediately after use.
    - Biohazard: Lead investigator must have been offered HEP B vaccination, and wear disposable gown and sterile gloves when handling the equipment, samples and contaminated materials. All sterile equipment must be handled by the lead investigator ONLY. Remove and

replace all collective clothing that gets contaminated with blood or bodily fluid as soon as possible. Clean any spillages with suitable disinfectant as per SOP. Participant that have or have partners that have known blood borne viruses should be excluded from the investigation.

- Summary of risks for participant
    - Chemical hazard: Injection of 1 % w/v Lidocaine local anaesthetic.
    - The biopsy procedure: The actual biopsy procedure can result in mild discomfort. The incision fully after ~10 days with minimal scarring. For ~2 days after the procedure the participant will experience localized stiffness which is quite normal.
    - Key safety issues for the participant (to minimize the above risks)
    - Chemical hazard: Injection of 1 % w/v Lidocaine local anaesthetic: precautions include: asking the participant of any heart problems, high or low blood pressure, liver problems, kidney problems, any allergies (in particular to localized anaesthetics).
    - Chemical hazard: Injection of 1 % w/v Lidocaine local anaesthetic: If the participant shows signs of an allergic reaction such as: feeling dizzy or restless, starts vomiting or has a ringing in their ears, blurred vision, starts twitching, has shallow breathing or slow heart rate, seek medical attention and refer them to A&E.
    - The biopsy procedure: intense physical exercise is not recommended during this period, but mobility is not affected. The participant will be monitored for several days after the biopsy procedure to ensure that the steri-strips and bandages are changed and the incision is healing.
    - In the event of the skin of the investigator being cut or penetrated by a needle or other sharp object which may be contaminated with blood or body fluids, or blood fluids enter the investigator through mucocutaneous exposure such as blood splashing into their eye or mouth:
        - IMMEDIATELY
        - Where appropriate wash splashes off your skin with soap and running water.
        - Where appropriate wash out splashes in the eyes using tap water or an eye wash bottle.
        - Where appropriate wash nose and mouth with plenty of tap water. DO NOT swallow the water.
        - If the skin is broken, encourage the wound to bleed and rinse thoroughly under running water. DO NOT suck the wound.
        - Record the course of contamination.
        - Seek medical advice.
    - Report as adverse incident according to appropriate SOP.

Appendix Four

General and Sport Nutrition Knowledge Questionnaire

Fill out the questions below to the best of your knowledge. Please **do not guess,** if you are unsure of an answer please tick the '**UNSURE**' box.

CARBOHYDRATE

1. In general are these foods **High** or **Low** in carbohydrate? *(Please tick one box per food).*

	High	**Low**	**Unsure**
Beef	☐	☐	☐
Pasta	☐	☐	☐
Cabbage	☐	☐	☐
Weetabix	☐	☐	☐
Chocolate spread	☐	☐	☐
Lentils	☐	☐	☐
Wholemeal bread	☐	☐	☐
Chicken	☐	☐	☐
Jelly babies	☐	☐	☐

2. In general as an athlete, what percentage of your diet should be made up from carbohydrate? *(Please tick one box)*

10 - 25 % ☐
25 - 40 % ☐
50 - 65 % ☐
65 - 80 % ☐
Unsure ☐

3. Do you know what the glycemic index is?

Yes ☐ No ☐

If you answer no, please go to question 7.

4. Which of the following phrases best describes the glycemic index? *(Please tick one box).*

The amount of carbohydrate a food contains ☐
The extent to which carbohydrate food raises blood sugar levels ☐
The extent to which protein food raises blood sugar levels ☐
The extent to which carbohydrate food raises blood pressure ☐
Unsure ☐

5. In general is it best to eat a diet rich in **High** or **Low** glycemic index carbohydrates *(Please tick one box).*

	High	Low	Unsure
	☐	☐	☐

6. Which of these foods are classified as **High** or **Low** in the glycemic index? *(Please tick one box per food).*

	High	Low	Unsure
Porridge oats	☐	☐	☐
Chick Peas	☐	☐	☐
Sweets (e.g. Gummi Bears)	☐	☐	☐
Dark chocolate (>70%coca)	☐	☐	☐
Honey	☐	☐	☐
Peanut butter	☐	☐	☐
Basmati rice	☐	☐	☐
Jacket potato	☐	☐	☐

7. Brown sugar is a healthier alternative the white sugar *(Please tick one box)*

True ☐ False ☐ Unsure ☐

8. A high carbohydrate diet helps to reduce protein breakdown in the body.

Agree ☐ Disagree ☐ Unsure ☐

PROTEIN

9. Would you **agree** or **disagree** with the following statements? (*Tick one box per question)*.

a) When lifting heavy weights the body uses protein as its main energy source.

Agree ☐ Disagree☐ Unsure ☐

b) Chicken is a very good source of energy to help fuel high intensity exercise.

Agree ☐ Disagree☐ Unsure ☐

10. Are the following foods **High** or **Low** in protein? *(Tick one box per food)*

	High	Low	Unsure
Chicken	☐	☐	☐
Kidney beans	☐	☐	☐
Potato	☐	☐	☐
Spaghetti	☐	☐	☐
Tuna	☐	☐	☐
Egg	☐	☐	☐
Peanut butter	☐	☐	☐
Spinach	☐	☐	☐
Cornflakes	☐	☐	☐
Malt Loaf	☐	☐	☐

11. What is the main use for protein in the body? (*Please tick one box)*.

Energy Source ☐
Growth and repair ☐
Improve hydration ☐
All of the above ☐
Unsure ☐

12. How much protein is there in the following food items? *(please tick one box per question)*

1 pint of skimmed milk

0.6 grams	6 grams	12 grams	40 grams	Unsure
☐	☐	☐	☐	☐

250 g tin of tuna

10 grams	20 grams	35 grams	50 grams	Unsure
☐	☐	☐	☐	☐

1 slice of white bread

0 grams	2 grams	4 grams	8 grams	Unsure
☐	☐	☐	☐	☐

13. In general how much protein (grams) should an average 70 kg male athlete eat per day? *(Please tick one box)*

49 – 77 grams ☐
105 – 119 grams ☐
154 – 196 grams ☐
217 – 266 grams ☐
Unsure ☐

14. There is more protein in a glass of whole milk compared to skimmed milk? (*True or false?).*

True ☐ False ☐ Unsure ☐

FAT

15. For improvements in health, what type of fat do experts recommend should be reduced in the diet? *(Please tick one box*).

Monosaturated fat	☐
Polyunsaturated fat	☐
Saturated fat	☐
Unsure	☐

16. Are these foods high in **saturated** or **poly-unsaturated** fat *(Please tick one box per food)?*

	Saturated	Poly - unsaturated	Unsure
Pumpkin Seeds	☐	☐	☐
Pork Chop	☐	☐	☐
Butter	☐	☐	☐
Olive Oil	☐	☐	☐
Mackerel	☐	☐	☐
Packet of Crisps	☐	☐	☐

17. Which of the following lipoproteins increase cholesterol? (HDL = high density lipoprotein) or (LDL = low density lipoprotein) (*Please tick one box)*

HDL's	☐
LDL's	☐
Unsure	☐

18. The following foods are high in cholesterol. (*Please tick one box per item)*

	True	False	Unsure
Coconut Oil	☐	☐	☐
Red Meat	☐	☐	☐
Eggs	☐	☐	☐
Walnuts	☐	☐	☐
Whole Milk	☐	☐	☐
Sunflower Seeds	☐	☐	☐
Sardines	☐	☐	☐

19. Do you think these foods are High or Low in fat? *(Please tick one box per item).*

	High	Low	Unsure
Orange	☐	☐	☐
Avocado	☐	☐	☐
Cottage cheese	☐	☐	☐
White bread	☐	☐	☐
Peanuts	☐	☐	☐
Sunflower seeds	☐	☐	☐
Jam	☐	☐	☐
Banana	☐	☐	☐

VITAMINS AND MINERALS

20. Do you **agree** or **disagree** with the following statements *(Please tick one box per question)*

    a) 500 ml of orange juice has the same number of calories as 500ml of orange squash.

    Agree ☐ Disagree ☐ Unsure ☐

    b) There is more calcium in a glass of whole milk than skimmed milk.

    True ☐ False ☐ Unsure ☐

21. What is the role of antioxidants in the body? (*Please tick one box)*

Help with energy production	☐
Help prevent against cell damage	☐
Increase metabolic rate	☐
Improve hydration status	☐
Unsure	☐

22. In General the following foods are naturally rich in antioxidant. *(True or false).*

	True	**False**	**Unsure**
Red Meat	☐	☐	☐
White Fish	☐	☐	☐
Fruit	☐	☐	☐
Vegetables	☐	☐	☐
Dairy	☐	☐	☐
Unsure	☐	☐	☐

23. If you want to eat something that is rich in Vitamin C, which of the following foods would you eat? (*Please tick one box per question*)

a)	Oranges	☐	Beef	☐	Unsure	☐
b)	Red pepper	☐	Spinach	☐	Unsure	☐
c)	Whitebait	☐	Baked beans	☐	Unsure	☐

24. If you want to eat something that is rich in iron, which of the following foods would you eat? (*Please tick one box per question*)

a)	Spinach	☐	Milk	☐	Unsure	☐
b)	Chickpeas	☐	Banana	☐	Unsure	☐
c)	All Bran	☐	Yoghurt	☐	Unsure	☐

25. If you want to eat something that is rich in calcium, which of the following foods would you eat? (*Please tick one box per question*)

a)	Spinach	☐	Chicken	☐	Unsure	☐
b)	Skimmed Milk	☐	Sunflower seeds	☐	Unsure	☐
c)	Potato	☐	Cheddar cheese	☐	Unsure	☐

26. Which cooking method is best to help maintain the vitamin and minerals content within vegetables. (*Please tick one box)*.

Steam	☐
Boil	☐
Fry	☐
Grill	☐
Unsure	☐

27. B vitamins are important for exercise because they help with: (*Please tick one box)*.

Hydration status	☐
Energy Production	☐
Immunity	☐
All of the above	☐
Unsure	☐

GENERAL NUTRITION

28. Cutting out 10 grams of carbohydrate from your diet will result in greater weight loss than cutting out 10 grams of fat. (*Please tick one box)*.

True ☐ False ☐ Unsure ☐

29. What food category are vegetables classified in? (Such as cabbage, carrots, onions, peppers, courgettes etc) (*Please tick one box).*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

30. During resting conditions, which is the predominant energy source the body uses? *(Please tick one box)*.

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

31. How many calories are there in 1 gram of each of the following macronutrients and Alcohol? (*Please tick one box)*

	Carbohydrate	Protein	Fat	Alcohol
2 kcal	☐	☐	☐	☐
4 kcal	☐	☐	☐	☐
7 kcal	☐	☐	☐	☐
9 kcal	☐	☐	☐	☐
13 kcal	☐	☐	☐	☐
Unsure	☐	☐	☐	☐

32. Does one glass of orange squash count towards the government recommended ‘5 a day’? (*Please tick one box)*

Yes ☐
No ☐
Unsure ☐

33. If you are trying to lose weight and want to have a snack what would be best the best food to snack on? (*Please tick one box per question)*.

a)	Peanut butter on a bagel	☐	**or**	Tuna sandwich	☐
b)	100 g chicken wrap	☐	**or**	100 g pork pie	☐
c)	150 g Tomato salad	☐	**or**	100 g rice pudding	☐
d)	Cottage cheese and berries (50 grams)	☐	**or**	Cheddar Cheese and crackers (50 grams	☐

Fluid

34. In general, how much fluid should you drink on average per day? (Please tick one box)

0 – 0.99 litres ☐
1 – 1.99 litres ☐
2 – 2.99 litres ☐
3 – 3.99 litres ☐
4 – 4.99 litres ☐
Unsure ☐

35. During exercise greater than one hour, what are the current guidelines for fluid consumption? *(Please tick one box).*

0 – 200 ml ☐
200 – 400 ml ☐
400 – 800 ml ☐
800 – 1,200 ml ☐
1,200 ml + ☐

36. In general, at what percentage of body dehydration would you start to see a decrease in exercise performance? (*Please tick one box).*

0.5 % ☐
2 % ☐
4 % ☐
6 % ☐
8 % ☐
Unsure ☐

37. A small amount of sodium added to fluid will help increase water absorption and improve hydration status? (*Please tick one box).*

True ☐ False ☐ Unsure ☐

38. In the first 2 hours after exercise how much fluid should you aim to drink?

10 % of sweat loss ☐
☐
☐
☐
☐

50 % of sweat loss
75 % of sweat loss
100 % of sweat loss
150 % of sweat loss

39. What type of drink would be best to consume in the following situations? *(Please tick one box per situation).*

	Hypotonic	Isotonic	Hypertonic	Unsure
a) Exercise lasting 0 – 45 minutes	☐	☐	☐	☐
b) Exercise lasting 45 – 90 minutes	☐	☐	☐	☐
c) Post exercise	☐	☐	☐	☐

40. How much carbohydrate is there in an isotonic sports drink? *(Please tick one box).*

0 – 3 % ☐
4 – 8 % ☐
8 – 11 % ☐
12 – 15 % ☐
Unsure ☐

41. The following drinks are isotonic. *(Please tick one box per question)*

	True	**False**	**Unsure**
a) Lucozade Sport	☐	☐	☐
b) Gatorade	☐	☐	☐
c) Red Bull	☐	☐	☐
d) Water	☐	☐	☐
e) Forgoodness Shakes	☐	☐	☐

SPORTING PERFORMANCE AND SUPPLEMENTATION

Please note, if you have not heard of any of the supplements listed below or are unsure of how they work, please tick the UNSURE box.

42. A high protein meal 1 hour before competing in a power event is recommended to enhance performance. *(Please tick one box)*

True ☐ False ☐ Unsure ☐

43. A high carbohydrate meal 2 – 4 hours pre exercise can lead to improvements in endurance performance. *(True or false?)*

True ☐ False ☐ Unsure ☐

44. Which of the following drinks contains the highest amount of carbohydrate? (P*lease Tick one box)*

500 ml Coke ☐
500 ml Powerade ☐
500 ml full fat milk ☐
500 ml orange squash ☐
Unsure ☐

45. In general, which of the following meals would be recommended to eat 3 hours before training? (P*lease Tick one box per question).*

a)	Steak and chips	☐	**or**	Cous cous and tuna ☐
b)	Mars bar and crisps	☐	**or**	Bread and jam sandwich ☐
c)	Chicken sandwich	☐	**or**	Cornish Pasty ☐
d)	Pasta and pesto	☐	**or**	Green leaf salad ☐

46. In general, what would be the best item to snack on in the 30 minutes pre exercise? *(Please tick one box per question).*

a)	Jelly babies	☐	**or**	Peanuts ☐
b)	Chocolate	☐	**or**	Banana ☐
c)	Sunflower seeds	☐	**or**	½ a white bread jam sandwich ☐
d)	Cereal Bar	☐	**or**	50g low fat crisps ☐

47. For a power athlete trying to increase muscle mass how much protein should they be eating per day (g/kg BM = grams per kilogram of body mass). *(Please tick one box)*.

0.5 – 0.9 g.kg BM ☐
1.0 – 1.4 g.kg BM ☐
1.5 – 2.0 g.kg BM ☐
2.1 – 2.5 g.kg BM ☐
2.6 – 3.0 g.kg BM ☐
Unsure ☐

48. If using a whey protein supplement, how much protein (in grams) do guidelines state should be consumed in one serving? *(Please tick one box)*.

10 – 17 g ☐
18 – 24 g ☐
☐
☐

25 – 30 g
31 - 37 g
38 – 45g ☐
Unsure ☐

49. If competing twice in one day, morning and evening:

a) When is the optimum time to eat after the first event? (*Please tick one box*).

0 – 45 minutes ☐
45 – 90 minutes ☐
90 – 125 minutes ☐
Eat after the second race. ☐
Unsure ☐

b) Is it more important to replace carbohydrate, protein or fat after the first event? *(Please tick one box)*.

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

50. When carbohydrate loading, what percentage of your diet should come from carbohydrate? *(Please tick one box)*.

45% ☐
60% ☐
75% ☐
90% ☐
Unsure ☐

51. Ideally, what percentage body fat would a world-class athlete have in the following events? *(Please tick one box per discipline)*

Sprinter	**Long Jumper**	**Marathon Runner**
0 – 3 % ☐	0 – 3 % ☐	0 – 3 % ☐
☐	☐	☐
☐	☐	☐
☐	☐	☐
☐	☐	☐

4 – 8 %	4 – 8 %	4 – 8 %
10 – 14 %	10 – 14 %	10 – 14 %
16 – 20 %	16 – 20 %	16 – 20 %
Unsure	Unsure	Unsure

52. Is it always beneficial to have a isotonic sports drink in the 60 minutes pre event? *(Please tick one box)*

Yes ☐
No ☐
Unsure ☐

53. What food group is made up of amino acid's? *(Please tick one box)*

Carbohydrate ☐
Protein ☐
Fat ☐
Unsure ☐

54. If an amino acid is termed 'essential' what does this mean? *(Please tick one box)*

You should consume this in your diet ☐
It is produced in the body ☐
Should eat it before you exercise ☐
Unsure ☐

55. Immediately post exercise is it best to consume high or low glycemic index carbohydrates? *(Please tick one box)*.

High GI ☐
Low GI ☐
Unsure ☐

56. Salt tablets could be used as a preventative if you regularly suffer from cramp during exercise. *(Please tick one box)*

Agree ☐ Disagree ☐ Unsure ☐

57. Supplementing with caffeine will result in a decrease in performance in the following events? (*Tick as many as relevant)*.

	True	False	Unsure
Sprinting	☐	☐	☐
Long Jump	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
		☐	☐

800 m
Shot Putt
Marathon
All or the above ☐

58. Supplementing creatine has the same effect as supplementing with whey protein (*Please tick one box)*.

True ☐ False ☐ Unsure ☐

59. In general, which athletic group would benefit from supplementing creatine monohydrate? (*Tick as many as relevant)*.

Speed and power ☐
Endurance ☐
Unsure ☐

60. If using creatine how much is recommended you should take per day? (*Please tick one box)*.

1 gram ☐
3 grams ☐
5 grams ☐
7 grams ☐
9 grams ☐
Unsure ☐

61. Which type of athlete, has research has shown, could benefit from taking sodium bicarbonate as a performance aid? *(Please tick one box)*

100 – 200 meters ☐
400 – 1500 meters ☐
5,000 – Marathon ☐
Throwers ☐
Unsure ☐

62. What are the physiological benefits of taking sodium bicarbonate? *(Please tick one box)*

Increase energy production ☐
Increase free radical removal ☐
Improve hydration status ☐
Maintain blood pH ☐
☐

Increase maximal voluntary contraction
Unsure ☐

Appendix Five

Recruitment adverts

Diet research

ATHLETES are invited to help in a research study into dietary habits and nutrition knowledge.

Those taking part by completing two short, online questionnaires and a three-day diet diary will receive a dietary analysis from sport scientists at the University of Hertfordshire. See http://sdu-surveys.herts.ac.uk/nutrition_knowledge

Male runner 18-35yrs w/ 5km PB <18 mins? Join our nutrition study. £600 inconvenience fee + GPS watch. Email hpl.contact@gsk.com - Pls RT

RESEARCH PARTICIPANTS NEEDED

A World Class human performance laboratory based in West London, who work with with elite athletes and the general population to further understand how to human body functions, is conducting a research study investigating the role diet plays on cellular and metabolic markers along with bone health. The Laboratory are ***looking for healthy male participants aged 18 – 35 years, who have no specific dietary requirements or allergies (e.g. not a vegetarian of Celiac).***

What will you receive for you participation?

- **£700 inconvenience payment.**
- **7 days food delivered to your door; prepared by Soulmate Food (www.soulmatefood.com)**
- **A comprehensive report detailing your metabolic rate, body composition and bone health.**

What is required from you?

- Attend a laboratory in West London on 3 separate mornings to undergo various tests at rest.
- Only consume the food that is delivered to you for the duration of the intervention (7 days) Food (www.soulmatefood.com).

Next steps

- **If you are interested, please contact Michael Porter on 01923 834239 or email mporter@medinovaresearch.com**

Ethics approval example

UNIVERSITY OF HERTFORDSHIRE
HEALTH & HUMAN SCIENCES

ETHICS APPROVAL NOTIFICATION

TO	Matthew Furber
CC	Dr Justin Roberts
FROM	Dr Richard Southern, Health and Human Sciences ECDA Chairman
DATE	02/10/14

Protocol number: **aLMS/PG/UH/00196**

Title of study: The impact of acute dietary macronutrient manipulation on cellular and metabolic biomarkers at rest

Your application to modify the existing protocol **LMS/PG/UH/00196** as detailed below has been accepted and approved by the ECDA for your school.

Modification: Modifications as described, and limited to, the notations on the EC1 which was attached with the EC2 request

This approval is valid:

From: 02/10/14

To: 01/08/15

Please note:

Any conditions relating to the original protocol approval remain and must be complied with.

Approval applies specifically to the research study/methodology and timings as detailed in your Form EC1 or as detailed in the EC2 request. Should you amend any further aspect of your research, or wish to apply for an extension to your study, you will need your supervisor's approval and must complete and submit a further EC2 request. In cases where the amendments to the original study are deemed to be substantial, a new Form EC1 may need to be completed prior to the study being undertaken.

Should adverse circumstances arise during this study such as physical reaction/harm, mental/emotional harm, intrusion of privacy or breach of confidentiality this must be reported to the approving Committee immediately. Failure to report adverse circumstance/s would be considered misconduct.

Ensure you quote the UH protocol number and the name of the approving Committee on all paperwork, including recruitment advertisements/online requests, for this study.

Students must include this Approval Notification with their submission.

Consent form example

Informed Consent Form

You have agreed to participate in a research study at the GSK Human Performance Lab (HPL), titled: Adapt and Perform

SESSION PROCEDURES: You hereby agree that you have had and understand all session procedures explained to you either orally or in writing. You have also read the *Research Participant Information Sheet* and had any questions answered to your satisfaction. During some assessment procedures you will be undertaking physical exercise at or near the extent of your capacity and there is possible risk in the physical exercise at that level, for example, episodes of transient light headedness, abnormal blood pressure, chest discomfort, and nausea. You understand that this may occur though the GSK HPL staff will take all proper care in the conduct of the assessment and you will fully assume that risk. Possible risk of certain blood borne diseases (HIV, Hepatitis B) is associated with any blood sampling that may occur and GSK HPL staff will take all proper care in the conduct of the blood sampling to reduce this. Where session procedures pose additional and specific risks, you will be provided with additional full written details to enable you to provide informed consent.

HEALTH: Health risks do exist when exercising. It is important that you notify the GSK HPL staff, and your trainer or team physician, of any unusual physical symptoms that develop during or after your time in the GSK HPL. You are required to complete the Medical Disclosure Form overleaf to inform GSK HPL staff of any illness or physical condition you have that may contribute to the level of risk. Information you disclose may be shared with your medical staff if deemed in the best interests of your health and/or performance.

HUMAN BIOLOGICAL SAMPLES: GSK represents and warrants that any human biological samples collected (blood, urine and muscle) in the GSK HPL will be obtained in material compliance with all applicable laws, regulations and any generally accepted ethical guidelines regarding the, collection, storage, transfer, subsequent use and disposal. You hereby provide informed consent that any human biological samples provided by you may be used for sport science and sports nutrition research purposes. Any remaining unused sample will be discarded once a specific piece of sport science or sports nutrition research has been completed. Storage of human biological samples by the GSK Human Performance Lab is in accordance with requirements of the UK Human Tissue Authority under HTA license number 12202. Any human biological tissue collected will be retained for ≤ 12 months. The HBS will be disposed of once analysed.

YOUR DATA: You agree that GSK (including, for the avoidance of doubt, GSK affiliates) may use, store and process your personal data for GSK HPL research and development or other purposes (including publication or marketing claims), but your personal identity will not be shared with third parties without your permission. You agree that GSK may share such data

with third parties for storage, processing and R&D purposes. You agree that personal data that you provide or which may be collected in the course of this study may be transferred to, stored and processed in your country of residence or any other country in which GSK or its affiliates, subcontractors or agents maintain facilities, including the United States and outside the European Economic Area ("EEA"). GSK is committed to using commercially reasonable means to ensure that your personally identifiable information is protected appropriately, and in accordance with applicable data protection laws, and uses a variety of security technologies and procedures to help protect your personally identifiable information from unauthorized access, use, or disclosure.

CONFIDENTIALITY: You shall maintain in strictest confidence any information acquired during your participation at the GSK HPL (including the location of the GSK HPL), whether that information relates to GSK, an affiliate or a third party, and there should be no disclosure to any third party of this information both during and after your participation.

PARTICIPATION: Your participation in this study is voluntary and you may refuse to participate or withdraw at any time without having to give reason, without penalty or loss of benefits to which you are otherwise entitled.

WAIVER: You acknowledge that your participation in this study is at your own risk and you have disclosed to GSK all relevant medical conditions. GSK, and third parties involved in the operation of the GSK HPL, shall be released from all liability to you with respect to any loss or damage suffered by you (in relation to injury, accuracy of the results or otherwise), to the fullest extent permissible by law. GSK does not attempt to limit its liability for death or personal injury caused by negligence.

Thank you.
SmithKline Beecham Limited, trading as GlaxoSmithKline Consumer Healthcare and its affiliates ("GSK")

Signed _____ (Participant

Date _____

Name _____

Authorisation
I am satisfied that the above-named has given informed consent to participate in a research study at the GSK HPL

Signed _____ (to be signed by HPL Principal Investigator)

Date _____

Name (Print) _____

Medical screen example

PRE-TEST MEDICAL Screen

Please answer the following questions as accurately as possible	Please tick	
	Yes	No
Has your doctor ever said that you have a heart condition *and* that you should only do physical activity recommended by a doctor?		
Are you suffering from any form of illness, or have you done so in the last 4 weeks? If yes, please specify what and when:		
Are you currently on any prescribed medication and/or have you taken any medication today? If yes, please specify:		
Are you suffering from any form of injury, bone or joint problem, or have you done so in the last 4 weeks? If yes, please specify what and when:		
Do you have exercise-induced asthma or exercise-induced bronchoconstriction? If yes, do you have your inhaler with you?		
Do you have any allergies? If yes, please specify:		
Do you have diabetes? If yes, please specify whether Type I or II:		

Do you have any blood disorder or infection (eg Haemophilia, Anaemia, Hepatitis, HIV/AIDS)? **If yes, please specify:**		
Have/Do you suffer from stress fractures? **If yes, please specify:**		
Have you ever had a Bone Density study (DEXA) done? **If yes, please specify:**		
Are there any other conditions or reasons that may prevent you from safely completing this testing session? **If yes, please specify:**		

I (print name) _____ confirm that the above information is accurate and that I am in a suitable condition to complete the performance assessment session at the GSK HPL. I will inform GSK HPL staff of any change in my condition at any future performance assessment sessions.

Signed _____ (to be signed by a parent / guardian if under 16)

Date _____

Authorised _____ (to be signed by HPL Scientist)

Muscle biopsy medical screen
To help us ensure your safety and wellbeing please answer the following questions.

1. Have you ever had a negative or allergic reaction to local anesthetic (e.g. during dental or surgical procedures)?
   No ☐ Yes ☐
2. Do you have any tendency toward easy bleeding or bruising (e.g with minor cuts or shaving)?
   No ☐ Yes ☐
3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Coumadin, Anti-inflammatories, Plavix)?
   No ☐ Yes ☐
4. Do you have any blood clotting or other disorders that you are aware of?
   No ☐ Yes ☐
5. Do you carry and EpiPen for any reason? Is so, please specify reason.
   No ☐ Yes_____
6. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?
   No ☐ Yes ☐
7. Have you ever had a muscle biopsy before, if so were there any complications?
   No ☐ Yes ☐
   Complications _____
8. Are you allergic to plasters?
   No ☐ Yes ☐
9. Will you contact the researcher who did the biopsy directly if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?
   No ☐ Yes ☐
10. Are you willing to visit the researcher who did the biopsy 7 – 10 days following the biopsy for an assessment of the biopsy site?
   No ☐ Yes ☐

Subject Name (print) :_____

Subject Signature :_____ Date :_____

Signature of Person Conducting Assessment:_____