An Evaluation of Nutritional Intake and Physical Activity during the British Army Officer Cadet Commissioning Course

Victoria Charlotte Edwards

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University of Chichester

Occupational Performance Research Group

Institute of Sport

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Abstract

Military training is conducted to develop individual and team skills in a progressive manner to prepare trainees to be able to perform their chosen job role. In undertaking these activities trainees are exposed to physically demanding tasks which will elicit physiological adaptations, intended to improve role-related fitness. However, long physically active days combined with sub-optimal nutritional intake can potentially be detrimental to health, impair performance and increase injury risk. The aim of the research presented in this thesis is to evaluate the nutritional behaviours of British Army Officer Cadets (OCs), in relation to their physical activity during military training, to inform evidenced based nutritional strategies to improve physical health and performance. The first four studies in this thesis report data collected during three different military training settings: on the academy grounds (CAMP; nine days), on field exercise (FEX; five days), and a training period that was a combination of the two (MIX; nine days). Study 1 quantified the Energy Intake (EI) and macronutrient intake of OCs during these settings. Energy intake was lower than thresholds in military nutritional guidelines and carbohydrate and protein intake were below athletic guideline thresholds. Study 2 showed that EI was lower than Energy Expenditure (EE) during training (indicating a negative Energy Balance; EB), however OCs body mass did not change over training (indicating EB). It has been suggested that body mass maintenance during negative EB may be a consequence of reduced metabolic functions in compensation for a lack of available energy. Study 3 estimated the Energy Availability (EA) of OCs during the same training periods and demonstrated EA to be low during times of field exercise, due to high EE and low EI. Study 4 showed that during FEX the distribution of energy and macronutrient (in particular protein) intake was suboptimal, perhaps reflecting sporadic eating due to limited time to eat. A nutrition intervention was therefore explored in Study 5 to investigate if two protein-rich supplements per day (217 kcal, 23.3 g protein, 13.6 g carbohydrate and 8.2 g fat; compared to a control group - no supplement) for 8 days could mitigate physical performance decrements associated with periods of negative EB during FEX. The FEX involved prolonged load carriage activity and resulted in a 2-day period of negative energy balance and low EA. The protein-rich supplement increased protein intake above athletic guidelines. However, pre- and post-FEX physical performance did not differ, irrespective of group. In summary, the research presented in this thesis is the first to quantify the nutritional behaviours of military personnel in relation to the physical demands across a range of military training settings which can be used to inform evidenced-based feeding interventions.

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Declaration of Authorship

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List of Abbreviations

ANOVA	Analysis of Variance
APRC	Army Personnel Research and Consultancy
BMR	Basal Metabolic Rate
CAMP	Camp Training
CC	Commissioning Course
CHO	Carbohydrate
COMA	Committee on Medical Aspects of Food Policy
CON	Control Group
d	Cohen's D
DLW	Doubly Labelled Water
DRVs	Dietary Reference Values
DXA	Duel Energy X-ray Absorptiometry
EA	Energy Availability
EB	Energy Balance
EB_{long}	Longitudinal Energy Balance
EE	Energy Expenditure
EI	Energy Intake
EIMD	Exercise-Induced Muscle Damage
FEX	Field Exercise
FFM	Fat Free Mass
FM	Fat Mass
g	Grams
h	Hour
IMTP	Isometric Mid-Thigh Pull
JSP	Joint Services Publication
kcal	Calories
kg	Kilogram
m	Meters
M	Meal
METs	Metabolic Equivalents
mg	Milligrams
MIX	Combined Camp and Field Training
ml	Millilitres
MTT	Military Task Training
MoD	Ministry of Defence
MoDREC	Ministry of Defence Research Ethics Committee
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
MDRVs	Military Dietary Reference Values
MSKI	Musculoskeletal Injury
mTOR	Mammalian Target of Rapamycin
OC(s)	Officer Cadet(s)
OPRG	Occupational Performance Research Group

PAEE	Physical Activity Energy Expenditure
PAL	Physical Activity Level
PRO	Protein
rCHO	Relative Carbohydrate
RDA	Recommended Dietary Allowance
RED-S	Relative Energy Deficiency in Sports
rFat	Relative Fat
RMAS	Royal Military Academy Sandhurst
RMR	Resting Metabolic Rate
rPRO	Relative Protein
S	Snack
s	Second
SACN	Scientific Advisory Committee on Nutrition
SKI	Ski March
SPSS	Statistical Package for the Social Sciences
SUP	Supplementation Group
TBW	Total Body Water
TDEE	Total Daily Energy Expenditure
TEF	Thermogenic Effect of Food
w	Watts
°C	Degrees Celsius
^{18}O	Heavy Isotopes of Oxygen
1RM	One-Repetition Maximum
^{2}H	Heavy Isotopes of Hydrogen
η^2_p	Partial Eta Squared

Overview of Projects

This programme of work was part of two larger projects funded by the UK Ministry of Defence (MoD) Army Personnel Research and Consultancy (APRC). This section describes the two larger projects and my unique role in the planning, data collection, analysis and interpretation for the work presented in this PhD thesis.

Studies 1 to 4 were part of a larger project funded through the Defence Human Capability Science and Technology Centre (DHCSTC). This larger project was conducted at the Royal Military Academy Sandhurst (RMAS [January 2017 to December 2017]) aiming to measure the physical activity profiles, nutrient intake and injury incidence in a cohort of Officer Cadets (OCs) over the duration (44 weeks) of the Commissioning Course (CC). The primary aims of this study were to, (1) develop a valid tool for measuring physical activity exposure in military personnel and (2) examine the association of physical demands of the CC with musculoskeletal injury risk in military personnel.

The target sample size of the larger overarching research project were 40 men and 10 women participants. These figures represented approximately one training platoon of male OCs and a sample of the female OCs in that current intake, which is representative of the female population of the British Army (~15%). Figure i shows the number of participants that were recruited for the larger research project (solid boxes), as well as the number of participants that withdrew during the year-long course. Due to these withdrawals, efforts were made to re-recruit when possible during the phases of research to maintain the sample size. Due to limited resources, a sub sample of 20 participants (dotted boxes), each term, volunteered to undertake extra physiological testing which were used in the data of the current thesis.

Researchers monitored OCs remotely, only visiting participants every 7 - 10 days, using wrist-worn activity monitors and weekly questionnaires on sleep quality, exertion, muscle soreness, physical activity, and injury incidence. Once during each of the three terms, a 10-day period of 'high resolution' monitoring was conducted where additional, more invasive monitoring was required; participants wore a chest-worn heart rate monitor, GPS monitor and researchers undertook daily observations of the OCs' physical activity. These blocks comprised solely training on camp (CAMP), solely training on field exercise (FEX), and a mix of camp and field exercise training (MIX). Within these 'high-resolution' blocks, a sub-sample of participants (10 men and 10 women personnel) were used to assess the validity of wrist-worn activity monitors and a physical activity log to measure Energy Expenditure (EE) against the gold-standard, criterion method, Doubly Labelled Water (DLW). This smaller cohort was also asked to provide daily food diaries, in addition to having all food from the dining hall / field kitchens weighed by the researchers, in order to estimate daily nutritional intake. My role in this larger project was as a researcher in a four-person team, where I collected and analysed low-resolution data on a weekly basis. I worked with the research team to plan the high-resolution data periods and took the lead role in the nutritional aspects of the data collection design, capture and analysis. This involved taking a leading role in the dosing of DLW, collection of urine samples, dietary weighing and nutritional analysis.

Study 5 was also conducted as part of a separate larger MoD funded project at RMAS in July 2019 to investigate: 1) sex differences in whole-body protein turnover and muscle function and 2) the effect of a dietary protein supplement on whole-body protein turnover and muscle function, before, immediately after, and 96 hours after a two-day field exercise. The study was conducted over a 10-day period during week 8 of the CC, which took place over Exercise Longreach - the most physically demanding exercise throughout the 44-week course. A total of 45 participants were recruited (15 control men, 15 control women, and 15 men who consumed two protein bars per day). The data from

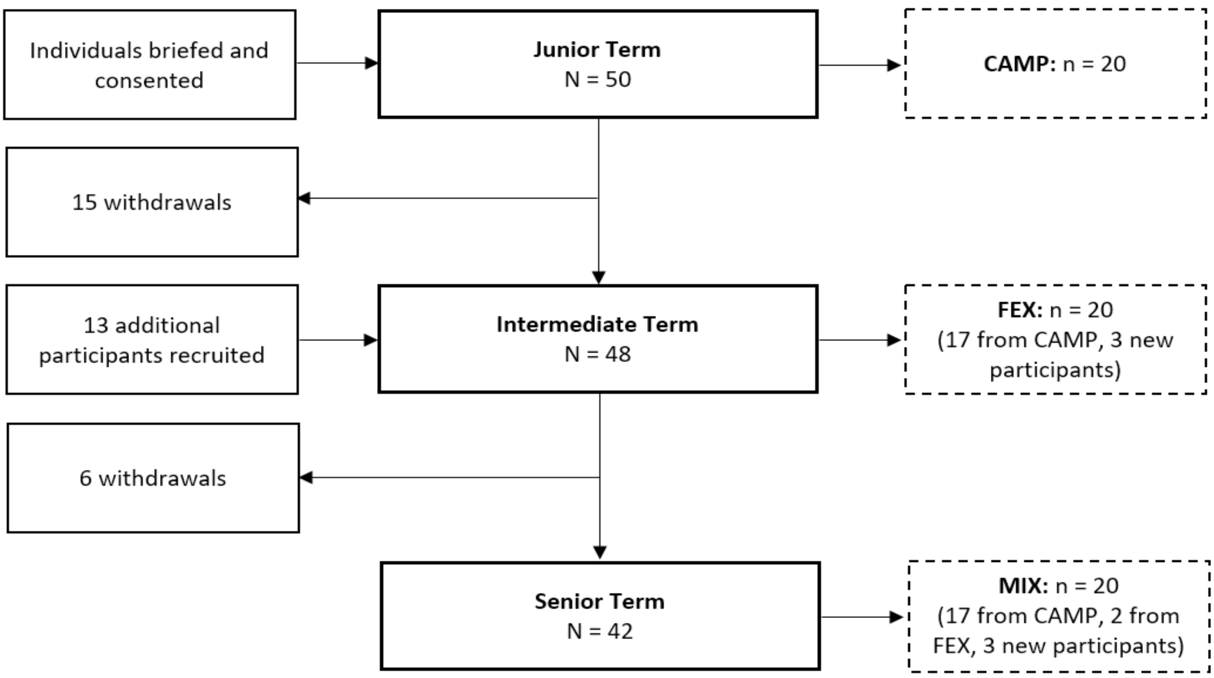

Figure i: *Schematic of volunteer recruitment and retention during Junior, Intermediate and Senior Term for the overall larger project (solid box) as well as for the high-resolution periods used in the current thesis (dotted boxes); camp training (CAMP), field exercise (FEX) and a combination of camp and field training (MIX)* the 15 control men and the 15 men who consumed two protein bars per day is presented in this thesis to investigate the effects of increased dietary protein intake in men during a field exercise.

As well as the measures presented in Study 5, the project involved measuring EE using the DLW method, protein turnover through ^{15}N-Glycine, and bone turnover through blood-borne biomarkers. I had a leading role in the project, working within the research team to design the project, leading in writing the Ministry of Defence Research Ethics Committee (MODREC) application, and presenting / defending the protocol at the MODREC meeting. During the field study, I took a leading role in several aspects of the project including the administration and analysis of GENEActiv activity monitors, collection and analysis of dietary intake, and muscular function measurements. I also assisted in leading the dosing of ^{15}N-Glycine and DLW, and the collection of urine and saliva samples for analysis. Due to circumstances that were out of the research group's control it was not possible for the data for protein turnover and DLW to be sufficiently analysed and be included in the current thesis.

List of Publications

Journal Articles in Preparation:

1. **Edwards, V. C**., Myers, S.D., Siddall, A. G., Needham-Beck, S. C., Powell, S. D., Jackson, S., Greeves, J. P., Wardle, S.L. & Blacker, S. D. Dietary Intake, Energy Balance and Energy Availability during British Army Officer Cadet Training (in preparation)
2. **Edwards, V. C**., Myers, S.D., Siddall, A. G., Needham-Beck, S. C., Powell, S. D., Jackson, S., Greeves, J. P., Wardle, S.L. & Blacker, S. D. The Daily Distribution of Dietary Intake during British Army Officer Cadet Training (in preparation)

Conference Abstracts

1. **Edwards, V.C**., Myers, S.D., Siddall, A.G., Needham-Beck, S.C., Powell, S.D., Jackson, S., Greeves, J.P., Wardle, S.L. & Blacker, S.D. (2020). Energy Availability of Officer Cadets during British Army Training. *5th International Congress on Soldiers' Physical Performance,* February 2020
2. **Edwards, V. C**., Myers, S. D., Siddall, A. G., Thompson, J. E., Powell, S. D., Jackson, S., Greeves, J.P., Wardle, S.L., Myers, S.D. & Blacker, S. D. (2018). Timing of Energy and Macronutrient Intake of British Army Officer Cadets during Military Training: 2623 Board# 5 June 1 3. *Medicine & Science in Sports & Exercise*, *50*(5S), 639. *(Thematic poster presentation at ACSM Annual Meeting 2018, 1st June 2018, Minneapolis, USA)*
3. **Edwards, V.C**., Siddall, A.G., Needham-Beck, S.C., Powell, S.D., Jackson, S., Greeves, J.P., Wardle, S.L., Myers, S.D., and Blacker, S.D. (2018). Timing of Nutritional Intake Around Physical Activity During Military Training. *3rd International Conference on Physical Employment Standards.* Portsmouth, UK. July 2018.
4. **Edwards, V.C.,** Siddall, A.G., Needham-Beck, S.C., Powell, S.D., Jackson, S., Greeves, J.P., Wardle, S.L., Myers, S.D., and Blacker, S.D. (2018). Timing of Dietary Intake of British Army Officer Cadets in Camp and Field Training Environments. *Sport Nutrition and Exercise Metabolism, 29*(Suppl1), S1-S16. (*Poster at the International Sport and Exercise Nutrition Conference, December 2018, Newcastle, UK).*

Publication from Projects Related to this Thesis

Papers

1. Siddall, A.G., Powell, S.D., Needham-Beck, S.C., **Edwards, V.C**., Thompson, J.E.S., Kefyalew, S.S., Singh, P.A., Orford, E.R., Venables, M.C., Jackson, S., Greeves, J.P., Blacker, S.D. & Myers, S.D. (2019). Validity of energy expenditure estimation methods during 10 days of military training. *Scandinavian Journal of Medicine & Science in Sports*, *29*(9), 1313-1321.

Conferences

1. Powell, S.D., Siddall, A.G., Needham-Beck, S.C., **Edwards, V.C.,** Jackson, S., Greeves, J.P., Blacker, S.D., Myers., S.D. Influence of Training Load on Injury Risk during British Army Officer Cadet Initial Military Training. (2020). *5th International Congress on Soldiers' Physical Performance*, February 2020

2. Siddall, A.G., Needham-Beck, S., Powell, S.D., **Edwards, V.C**., Blacker, S.D. Jackson, S., Greeves, J.P. & Myers, S.D. (2020). Accuracy of activity zone classification between heart rate reserve and accelerometry in military field exercise. *5th International Congress on Soldiers' Physical Performance*, February 2020

3. Blacker, S. D., Siddall, A. G., Needham-Beck, S., Powell, S. D., **Edwards, V. C**., Kefyalew, S.S., Singh, P.A., Orford, E.R., Venables, M., Jackson, S., Greeves, J.P. & Myers, S.D. (2019). Equation to Estimate Total Energy Expenditure in Military Populations Using a Wrist-Worn Physical Activity Monitor: 1039: Board#273 May 29 2:00 PM-3: 30 PM. *Medicine & Science in Sports & Exercise*, *51*(6), 275-276.

4. Powell, S.D., Siddall, A.G., Thompson, J.E.S., Needham-Beck, S.C., **Edwards, V.C**., Jackson, S., Greeves, J.P., Blacker, S.D., Myers., S.D. (2018). *Gender Differences in Cardiovascular Strain During British Army Officer Cadet Training. Presented at the 3rd International Conference on Physical Employment Standards, July 2018, Portsmouth, UK.*

5. Needham-Beck, S.C., Siddall, A.G., Thompson, J.E., Powell, S., **Edwards, V.C**., Blacker, S.D., Jackson, S., Greeves, J.P. & Myers, S.D. (2018). Comparison of Training Intensity, Energy Balance, and Sleep Duration in British Army Officer Cadets between Base and Field Exercise: 2622 Board #4. *Medicine & Science in Sports & Exercise*, *50*(640)

6. Powell, S., Siddall, A.G., Thompson, J.E., **Edwards, V.C**., Jackson, S., Greeves, J.P., Wardle, S.L., Blacker, S.D. & Myers, S.D. (2018). Comparison of Daily Energy Expenditure and Weekly Physical Activity Exposure Estimated Using Consumer and Research-grade Physical Activity Monitors During Officer Cadet Initial Military Training: 2625 Board #7. *Medicine & Science in Sports & Exercise, 50*(640)

7. Siddall, A. G., Thompson, J.E., Powell, S. D., **Edwards, V.C**., Kefyalew, S.S., Singh, P.A., Orford, E.R., Venables, M., Jackson, S., Greeves, J.P., Blacker, S.D. & Myers, S.D. (2018). Comparison of Research- and Consumer-grade Energy Expenditure Estimation Methods during 10 Days of Military Training: 2624 Board #6. Medicine & Science in Sports & Exercise, *50*(640)

8. Siddall, A., Thompson, J.E., Powell, S.D., **Edwards, V.C.**, Blacker, S.D., Jackson, S., Greeves, J.P & Myers, S.D. (2017). Estimation of military training demand from a wrist-worn activity monitor and the potential impact of activity level on improvement of aerobic fitness. *Journal of Science and Medicine in Sport*, *20*, S25.

Chapter 1

Introduction

Introduction

The physical activity conducted during military training (external workload) and associated physiological strain (internal workload) should provide an appropriate stimulus to improve physical fitness but not be so excessive that it unduly increases risk of injury and illness. High physical activity levels are common during military training (Margolis, Crombie, et al., 2014; Richmond et al., 2012; Tharion et al., 2005; Wilkinson et al., 2008). This physical activity includes military tasks which are often performed carrying external loads and are coupled with multiple stressors such as physiological strain, fatigue, calorie restriction and sleep deprivation (Henning et al., 2011; Pihlainen et al., 2017). Long physically active days in training are used to impose physical and psychological stress upon personnel, in order to prepare them for the demands of combat (Tharion et al., 2005), and to elicit physiological adaptations to improve role-related fitness (Pasiakos & Margolis, 2017). However, these combined stressors encountered during training can potentially be detrimental to health, impair performance and increase injury risk (Knapik et al., 2001). These negative consequences have shown to be a leading health and readiness concern for Armed Forces (Bullock et al., 2010; Jones et al., 2010). Therefore, a critical goal of military training is balancing the need to attain, and maintain, a high level of physical fitness whilst minimising injury risk (Knapik & Reynolds, 2010).

In the military, long periods of sustained low-to-moderate physical activity and intermittent sleep deprivation, particularly during field training exercises and operations, can result in high daily Energy Expenditure (EE) that are difficult to match with Energy Intake (EI) (Margolis et al., 2013; Nindl et al., 2007). Energy expenditure has been reported to be as high as 5091 $kcal\cdot d^{-1}$ in British Army soldiers during a military training course, 5480 $kcal\cdot d^{-1}$ for Norwegian soldiers when training in camp and up to 6851 $kcal\cdot d^{-1}$ during field exercises in cold weather conditions (Margolis, Murphy, et al., 2014). In settings where soldiers experience high EE, their EI should aim to be sufficient to support the demands of training, and as such, nutritional recommendations have been produced for military personnel (SACN, 2016).

Figure 1.1 presents a theoretical model of the interaction between external and internal workload and training outcomes in the military setting, which collectively contribute to operational effectiveness. There are a number of modifiable and non-modifiable factors which affect the physiological responses to the external workload and resultant training outcomes, one of which is nutrition. In theory, optimising nutritional intake through evidence-based interventions should enable soldiers to better tolerate the high external workloads associated with military training and result in improved functional adaptation, reduced musculoskeletal injury (MSKI) and higher retention, resulting in greater overall operational effectiveness.

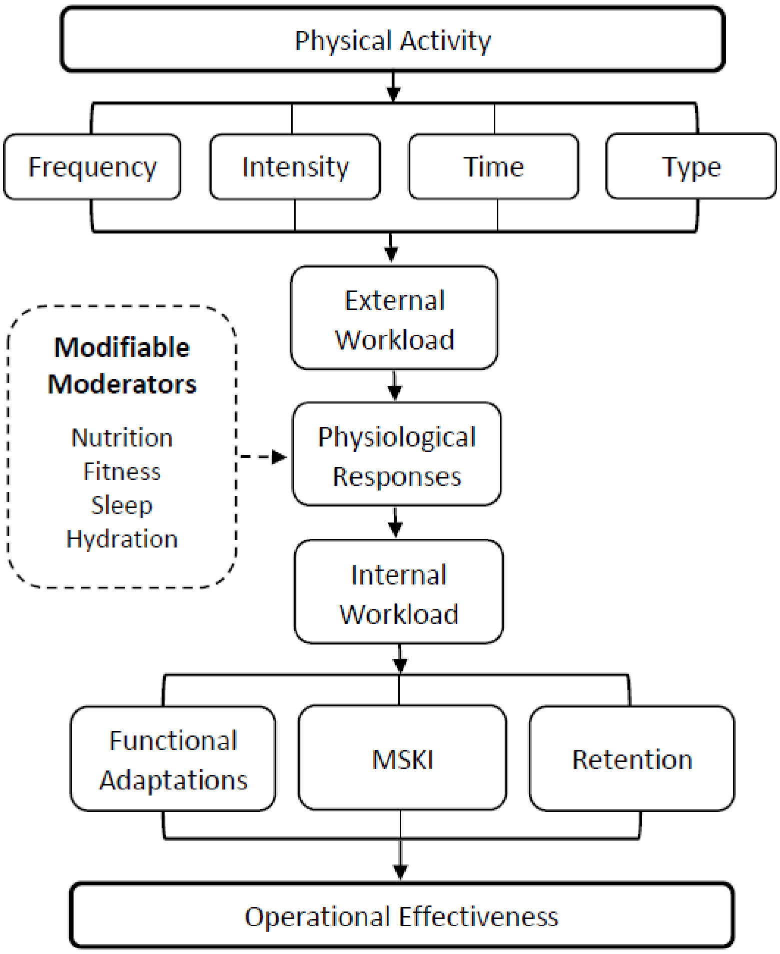

Figure 1.1: *A conceptual diagram of the external and internal workloads that, when optimised, can result in greater overall operational effectiveness*

In the UK Armed Forces nutritional guidelines provide information on appropriate energy, macronutrient and micronutrient intake (Hill et al., 2011). Sex-specific Military Dietary Reference Values (MDRVs) during Officer Cadet (OC) training have been proposed for energy, with EI guidelines of 4600 and 3500 kcal·d^{-1} for men and women, respectively (SACN, 2016). Different requirements are given for different job roles, such as administration-based roles in the active service, which are much less active, are recommended to consume 3600 and 2800 kcal·d^{-1} (50th percentile) for men and women, respectively. The UK macronutrient guidelines for military personnel are largely based upon evidence from athletic populations undertaking sports training and competition, and on providing sufficient energy to meet demands of the training which are set as a percentage of EI (SACN, 2016). Recommendations are provided as a range for carbohydrate (55 - 65% of total EI), protein (10 - 15%

of total EI) and fat (25 - 35% of total EI), with a lower limit set to reflect the recommendations of the general population (SACN, 2016). However, more recent reviews in the sports performance literature have suggested that this is not the most desirable way of expressing intakes and perhaps carbohydrate and protein guidelines should be expressed per kilogram of body mass (Burke et al., 2001; Phillips, 2012). Guidelines for recommended carbohydrate intake range between 3 - 12 $g \cdot kg \cdot d^{-1}$ for athletes, with the higher recommendations important to support moderate-to-high intensity exercise (> 4 - 5 $hours \cdot d^{-1}$) (Potgieter, 2013). Low carbohydrate intake has been shown to impact soldier performance on military tasks (Jacobs & Sherman, 1999; Montain et al., 1997), and thus high carbohydrate intakes are likely to be necessary to enhance recovery, optimise glycogen stores for the next training session (Potgieter, 2013), and enhance / maintain performance when undertaking endurance exercise, high intensity interval training and long duration field exercises (DeBolt et al., 1988).

The current UK Government Recommended Dietary Allowance (RDA) for protein intake in the general population is 0.8 $g \cdot kg \cdot d^{-1}$ (Gillen et al., 2017; IOM, 2000). However athletes involved in strenuous exercise training, and military personnel undergoing arduous training, may require protein intakes up to twofold higher than the recommended guidelines, with suggestions of 1.2 - 2.0 $g \cdot kg \cdot d^{-1}$ (Pasiakos, Montain, et al., 2013; Phillips et al., 2007; Phillips & Van Loon, 2011; Rodriguez et al., 2009). Protein intake at the higher end of the recommendation may optimise muscle gain, increase strength and power, and aid muscle recovery (Phillips & Van Loon, 2011; Rodriguez, 2013; Rodriguez et al., 2009). A higher protein intake has also been found to mitigate declines in whole-body protein balance, with protein consumption at twice the recommended allowance promoting protein balance during short-term moderate energy deficit (Margolis, Murphy, et al., 2014; Pasiakos, Austin, et al., 2013). Importantly, energy restriction, such as occurs in military field exercise, may increase the needs of protein intake beyond even that recommended for athletes (2.0 - 3.0 $g \cdot kg \cdot d^{-1}$) (Helms et al., 2014). This higher intake may be needed to support adaptations to training and counter the negative effects of nutritional deficit, such as loss of muscle mass, insufficient muscle recovery and detriments to performance (Areta et al., 2014; Etheridge et al., 2008; Fortes et al., 2011; Hector et al., 2017; Pasiakos, Cao, et al., 2013).

During military field exercises and operations, combat ration packs are provided to military personnel when it is not practical to establish a field kitchen to provide fresh food (Tassone & Baker, 2017). The ration packs contain a mixture of packaged food and drink items including main courses, snack items and beverage powders, which, when used exclusively and all items are consumed, are designed to provide military personnel with appropriate nutrition (Tassone & Baker, 2017). The energy provision of ration packs, are based on the MDRVs, and provide 4000 $kcal \cdot d^{-1}$ for Army personnel during training, exercises and operations (Davey et al., 2013). A UK Armed Forces ration pack typically consists of 651 g carbohydrate, 130 g protein, and 92 g fat (Shaw & Fallowfield, 2013), which corresponds to the upper end of the MDRVs for carbohydrate (65 %) and mid-range for protein (13%) and fat (20%).

The dietary habits and nutrient intake of military personnel, especially during Army OC training, are largely unknown. Previous studies on other UK Armed Forces training courses, have found Royal Navy OCs consume less than the recommended guidelines for energy and macronutrient intake (Fallowfield et al., 2011). During times of arduous training, Army personnel are often unable to match the required EI (Margolis, Murphy, et al., 2014; McAdam et al., 2018; Richmond et al., 2014), irrespective of whether they are provided with an adequate amount of food, however this has not been explored in OCs during British military training. Likewise, during military exercises, although rations are designed to provide appropriate nutrition, military personnel often have insufficient time to prepare meals to eat, and often 'strip' rations of items they do not want (Tassone & Baker, 2017). Therefore, while adequate food may be supplied, actual dietary intake may be insufficient to meet the demands of military training.

Periods of high EE coupled with low EI are common in military training and field exercises and can lead to periods of negative Energy Balance (EB), which can subsequently result in a loss of body mass. A negative EB has been shown to adversely impact health (Carbuhn et al., 2010), increase injury rates (Dixon & Fricker, 1993; Schlabach, 1994), and compromise physical performance (Montain & Young, 2003). These effects are most likely due to the resulting loss in body mass, which is accounted for by 60 - 80% of Fat Mass (FM) (Hill et al., 2013), and up to 25% of Fat Free Mass (FFM) (Hector et al., 2017). During a period of energy deficit, whole-body protein breakdown and whole-body protein synthesis are reduced, with synthesis being reduced to a greater degree, conserving energy and endogenous protein reserves (Pasiakos, Margolis, et al., 2014; Stein et al., 1991), and therefore creating a state of negative protein balance. However, the effect of negative EB on whole-body protein turnover varies depending on the degree and length of the energy deficit period (Pikosky et al., 2008).

Edholm and Fletcher (1955) proposed that small day-to-day variations in EB are unlikely to result in significant changes in body mass that would be detrimental to performance, since it is typical for EI to be naturally matched by free-living EE in the intervening and subsequent days. It is likely that any changes in body mass over periods of 3 - 7 days are primarily caused by water loss (Margolis et al., 2016; Margolis, Murphy, et al., 2014). However, decreases in body mass over longer training periods (weeks-to-months) of near continuous activity will more likely be a consequence of negative EB and are considered to be more detrimental to both physical and cognitive performance (Friedl, 1995; Pasiakos & Margolis, 2017).

Although the model of EB has been the usual basis for research and practice (Loucks et al., 2011), it assumes that all physiological functions are at an optimal level, which may not always be the case (Papageorgiou, Dolan, et al., 2017). It has been proposed that during an energy deficient state, Basal Metabolic Rate (BMR) may be reduced in an attempt to restore EB with the suppression of non-immediately essential physiological functions (Svendsen et al., 1993; Wade et al., 1996) including, but not limited to, metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Hackney, 2020). This concept is termed low Energy Availability (EA) and refers to the mismatch between EI and Exercise EE or daily work, leaving inadequate energy to support the functions of the body to maintain optimal health and performance (Mountjoy et al., 2018). Low EA can occur through a reduction in EI and / or increased exercise load, leading to disruption and / or impaired physiological and metabolic function (Mountjoy et al., 2014). Although no studies have investigated low EA in the military directly, one study found soldiers had a decrease in endocrine function during a period of energy deficit (-1800 $kcal \cdot d^{-1}$) during a combat course (Gomez-Merino et al., 2002). Despite the increase in awareness of low EA in athletic settings, little is currently known about the time-frame in which low EA can have a detrimental effect on health and performance.

The negative consequences of suboptimal EB and EA have been shown to be mitigated by nutritional strategies. For example, additional dietary intake of carbohydrate and protein may significantly affect the adaptive response to exercise and mitigate effects of negative EB (Kerksick et al., 2008). During military training, low-to-moderate intensity exercise can produce severe energy deficits that can deplete endogenous carbohydrate stores (Margolis, Murphy, et al., 2014). This low carbohydrate state can trigger essential metabolic alterations to mobilise endogenous lipids and preserve endogenous carbohydrate, thus suppressing carbohydrate oxidation (Horowitz et al., 2005). Suboptimal intake of carbohydrate results in decreased power output and early onset of fatigue (Beals et al., 2015). Although no studies have directly measured the impact of low carbohydrate on military performance, it has been suggested that sub-optimal carbohydrate intake could negatively affect rifle shooting performance and muscle function (Blacker, Williams, et al., 2010; Tharion & Moore, 1993). Additionally, consuming additional carbohydrate during, and after, exercise has resulted in faster recovery of neuromuscular function following military load carriage (Blacker, Williams, et al., 2010). A combined intake of carbohydrate, protein and fat has also been shown to increase the rate of Muscle Protein Synthesis (MPS) and attenuate the rate of Muscle Protein Breakdown (MPB) after exercise (Elliot et al., 2006), and therefore would be beneficial during times of negative EB.

Increasing dietary protein intake during periods of negative EB can also be an effective countermeasure to attenuate muscle protein loss, thus protecting muscle mass and reducing the consequential negative effects associated with negative EB (Montain & Young, 2003). Previous work has demonstrated that increasing protein intake to twice the RDA (1.6 $g \cdot kg \cdot d^{-1}$) during an energy deficit can increase Muscle Protein Synthesis (MPS) and attenuate the loss of FFM (Pasiakos, Cao, et al., 2013). Protein intake has also been shown to be necessary for the repair and remodelling of the skeletal muscle after exercise (Beelen et al., 2010), which is critical given that military training, such as load carriage, has been shown to induce muscle damage (Blacker, Williams, et al., 2010; Margolis, Murphy, et al., 2014).

Although the total intake of protein is important to maintain protein balance, the timing of dietary protein intake can also play an important role. Considerable work has been undertaken to determine the optimal timing of nutritional intake in order to maximise post-exercise MPS and promote adaptations to training (Atherton & Smith, 2012; Cribb et al., 2006). These studies have shown that when multiple doses (4 x 20 g) of protein were consumed over 12 hours, MPS was greater compared to two 40 g boluses throughout the day (Areta et al., 2013). The timing of protein intake, has been explored in both controlled laboratory and sports performance settings, where typically macronutrient intake is manipulated around a single bout of exercise to determine the influence on performance, MPS or muscle damage (Areta et al., 2013; Nosaka et al., 2006; Roy et al., 2000). Therefore, whilst military training is not solely focused on resistance training or endurance exercise performance, the application of these principles may be useful in the maintenance of muscle mass, protection against glycogen depletion and for optimising exercise recovery.

In the military setting further research is needed to quantify the patterns of nutritional intake in relation to the physical activity during military training. This information can then be used to inform evidence-based nutritional interventions that could potentially improve training outcomes and operational effectiveness. Therefore, the aims of the research presented in this thesis are to:

1. Quantify the physical activity profile of the 44-week Commissioning Course (CC) and determine the energy and macronutrient intake of OCs during training compared to current military and athletic guidelines (Study 1 - Chapter 4);
2. Quantify longitudinal and acute EB in OCs during the 44-week CC and to determine whether estimated body mass change agrees with actual body mass change over the acute periods (Study 2 - Chapter 5);

3. Investigate EA in OCs undertaking arduous training during the 44-week CC (Study 3 - Chapter 6);
4. Determine differences in the daily distribution of nutritional intake in relation to physical activity in three different military settings; on camp only, during field exercises and combined camp and field training (Study 4 - Chapter 7);
5. Investigate the effect of dietary protein supplementation on energy and macronutrient intake and muscle function and soreness during an arduous military field exercise (Study 5 - Chapter 8).

Chapter 2

Literature Review

Literature Review

This review of literature will first summarise the physical demands of military training, focusing on the high Energy Expenditure (EE) observed during camp-based training and field exercises. Nutritional guidelines for military personnel and athletes that are recommended to maintain optimal health and wellbeing of personnel are then reviewed. Physical activity and nutritional intake are then collectively considered to discuss the implications of Energy Balance (EB) and Energy Availability (EA) within military settings, and the importance of nutritional intake and timing during times of high EE. Finally, feeding strategies to enhance training adaptations and recovery in military settings are then summarised.

2.1 Physical Demands of Military Training

Military personnel train to ensure constant mission and deployment readiness (Nindl et al., 2013). Soldiers must develop, and maintain, high levels of physical fitness throughout their career which are comparable to that of an athlete training for competition (Jones & Knapik, 1999). Higher levels of physical fitness are likely to improve physical performance, make personnel more resilient to operational stressors and reduce risk of injury (Henning et al., 2011).

Training programs are designed to be progressive and result in physiological strain which is adequate to improve fitness (Pollock et al., 1998), without being detrimental to health (Haskell et al., 2007; O'Donovan et al., 2010). High physical activity levels are common during military training (Bilzon et al., 2006; Margolis, Crombie, et al., 2014; Richmond et al., 2012; Tharion et al., 2005; Wilkinson et al., 2008), where military tasks are often performed carrying external loads and are coupled with multiple stressors, such as physical strain, fatigue, energy restriction and sleep deprivation (Henning et al., 2011; Pihlainen et al., 2017). Additional to the physiological strain imposed on soldiers, such as load carriage, cognitive load such as communicating and decision making, can be placed on the soldiers independently or simultaneously to physiological load (Qu, 2013). Long physically active days in training are used to impose physical and psychological stress upon soldiers to prepare them for combat (Tharion et al., 2005). These stressors combined can potentially impair physical and mental performance, which may be detrimental to combat effectiveness. Knapik et al. (2001) demonstrated that being less physically active prior to training and having a relatively larger increase in training load increases injury risk. Therefore, a critical goal of military training is balancing the need to improve and maintain a high level of fitness whilst minimising the chance of injury. As illustrated previously in Figure 1.1 in Chapter 1, one modifiable factor that can affect the physiological and psychological responses to a high workload, is nutrition. Nutrition is essential for individuals to meet, and adapt to, the demands of training (Beals et al., 2015), to ensure physiological mechanisms are functioning optimally (Stubbs et al., 2004), to enhance and / or maintain physical and cognitive performance (McClung & Gaffney-Stomberg, 2016; Moran et al., 2012) and to reduce risk of fatigue, injury and illness (Rodriguez et al., 2009). Reducing negative EB and mitigating low EA should enable soldiers to better tolerate the high external workloads associated with military training and should notionally result in improved functional adaptation, reduced MSKI and higher retention, resulting in greater overall operational effectiveness. Despite the importance of understanding the cognitive load and the effect nutrition may have in improving this stressor, it was not within the remit of this body of work and therefore will not be explored.

2.1.1 *The British Army Officer Commissioning Course at the Royal Military Academy Sandhurst*

The research presented in this thesis has been conducted during Officer Cadet (OC) training at Royal Military Academy Sandhurst (RMAS). The RMAS Commissioning Course (CC) is a total of 44 weeks and is broken down into three 14-week terms, Junior, Intermediate and Senior Term, where each is separated by one week of adventure training and two to three weeks of leave. The course is necessarily arduous, where men and women are trained together in integrated platoons, to master military skills through classroom and practical lessons, and to improve physical fitness. Multiple modules of the course must be successfully completed before the OCs receive their commission. The CC is usually followed by further training courses specific to the Regiment or Corps in which the Officer will serve.

The CC is cognitively and physically demanding and RMAS is one of the most revered military training academies worldwide. The CC involves both on-camp training as well as UK and overseas exercises. On camp, OCs will train during the day around the Academy, eat in the dining facility and sleep in their accommodation lines. Field exercises that are situated both in the UK and overseas can involve sleeping in tents, shelters or in the open, during varying environmental conditions, and include periods of intense physical training, emotional and mental stress, and periods of sleep deprivation. Physical fitness is developed through physical training lessons, which include activities such as plyometric exercise, interval training, resistance and endurance training, and self-selected sports on a weekly basis. Simulated tasks include drills[1], loaded and unloaded marches, prolonged physical activity, and lifting / carrying load.

2.1.2 *Energy Expenditure during Military Training*

High EE is experienced during military training due to the continuous nature of work and training consisting of intermittent periods of moderate-to-high intensity activity, combined with periods of prolonged low intensity exercise (Henning et al., 2011; Skiller et al., 2005; Tharion et al., 2005; Wilkinson et al., 2008). This pattern is often seen in military training courses, where students can be physically active for 16 - 22 hours a day (Fairbrother et al., 1995; Hoyt et al., 2001; Tharion et al., 2004; Tharion et al., 2005). This has been demonstrated previously in British Army OCs at RMAS where EE was shown to be as high as 4898 ± 430 in men and 3822 ± 478 kcal·d^{-1} in women (Bilzon et al., 2006). During these times, military personnel are still required to maintain high levels of alertness, attention, executive function and job performance (Alemany et al., 2008; Lieberman, 2003; Pasiakos et al., 2015).

Table 2.1 summarises previous literature measuring the EE of military personnel on both camp and field exercise / operations, with the highest EE (6851 ± 562 kcal·d^{-1}) reported in Norwegian Conscript soldiers participating on a 3-day ski march (Margolis, Murphy, et al., 2014), and Norwegian Army Ranger Cadets (6358 ± 478 kcal·d^{-1}) on a 7-day field exercise, reported to be due to sleep deprivation and periods of sustained activity (Hoyt et al., 2006). The large differences in EE between studies are likely due to the varying natures of the different training courses measured, the environmental conditions in those situations and the tasks involved. Although extremely high EE was reported in Margolis, Murphy, et al. (2014), this was most likely due to the environmental conditions and workload, where soldiers were undertaking field training tasks at -15°C in Norway and skiing for 6 - 10 hours per day carrying ~45 kg, as opposed to lower EE reported in the same study during the in-camp training where soldiers carried out classroom based activities. Lower EE was also reported in Edholm et al. (1970) where soldiers were involved in more classroom based activities within a UK-based training camp. Field environments have previously been shown to elicit higher EE compared to camp due to the amount of ambulatory activities, carrying heavy load and longer workdays (Tharion et al., 2005). Hoyt et al. (2006) reported high EE (5231 ± 478 kcal·d^{-1}) during a field exercise, measured via Doubly Labelled Water (DLW), in women Norwegian Cadets, which, although lower than their male counterparts (6355 ± 478 kcal·d^{-1}), showed no difference when accounting for differences in body

[1] Training in marching and the use of weapons

mass. In the few studies that have reported EE in women, EE can be comparable to men when accounting for differences in body mass (Bilzon et al., 2006; Hoyt & Honig, 1996; Hoyt et al., 2006; O'Leary et al., 2018) and lean body mass (Tharion et al., 2001). O'Leary et al. (2018) reported that during weeks 1 and 2 of British Army basic training, absolute EE was different between men and women, explained by the differences in body mass, however relative EE was no different. Additionally, the authors reported that during week 12 and 13 of the same course, the relative EE in men was 12% higher compared to women. This was likely explained by the greater emphasis on load carriage in the latter weeks and therefore, due to the lower body mass of women, caused a greater physiological strain (O'Leary et al., 2017). Load carriage, ranging from 30 - 60 kg, is a contributing factor to the high metabolic cost of military training, substantially increasing the physiological strain of locomotion, because oxygen consumption, heart rate and ventilation are increased and endurance capacity is markedly less (Grenier et al., 2012; Ricciardi et al., 2008).

The EE of military personnel is higher during training than the general population, but comparable to those seen in athletic populations and thus research on the athletic population will be used to explore nutritional strategies in the current body of work. For example, the EE of male rugby league players during their competitive season was reported to be 4369 ± 979 $kcal \cdot d^{-1}$ over 14 days (Smith et al., 2018) measured by the Doubly Labelled Water (DLW) technique. Likewise, male cyclists training for the Tour de France, had an average EE of 4562 ± 979 $kcal \cdot d^{-1}$ over six days (Vogt et al., 2005) estimated through a cycle ergometer recording heart rate. Elite female distance runners have been shown to have an EE of 2991 ± 415 $kcal \cdot d^{-1}$ over seven days, measured via DLW (Edwards et al., 1993), and an EE of 3957 ± 1219 $kcal \cdot d^{-1}$ over 14 days has been reported in female rowers (Hill & Davies, 2002). Although the demands of many sports can vary compared to military training, the periods of prolonged intense exercise, that aim to progress towards a specific goal over many weeks while attempting to maintain or attain favourable body composition for performance such as that experienced by cyclists and other endurance-based athletes, has its similarities to that of military personnel. Therefore, studies investigating nutritional demands and strategies in the athletic population may be a valuable guideline when understanding the nutritional requirements of soldiers.

Table 2.1: *Previously reported Energy Expenditures (EE) of Men (M) and Women (W) personnel undergoing military training courses published in the peer-reviewed literature*

Reference	Population	Training Duration & Type	Measurement	*n*	Sex	EE (kcal·d^{-1})
Edholm et al. (1970)	British Infantry Soldiers	7-day Infantry training course	Indirect calorimetry	35	M	3750 ± 399
Burstein et al. (1996)	Israeli Infantry Soldiers	12-day winter camp	DLW	18	M	4281 ± 170
Burstein et al. (1996)	Israeli Infantry Soldiers	12-day summer camp	DLW	12	M	3937 ± 159
Hoyt et al. (2006)	Norwegian Army Cadets	5 to 7-day exercise	DLW	10	M	6358 ± 478
				6	W	5234 ± 478
Bilzon et al. (2006)	British Army Officer Cadets	Three 2-week periods during camp training	DLW	8	M	4782 ± 502 - 4898 ± 430
				8	W	3775 ± 454 - 3822 ± 478
Wilkinson et al. (2008)	British Parachute Regiment	10-day combined Infantry training course	DLW	20	M	4553 ± 571
Blacker et al. (2011)	Gulf Cooperation Council Officer Cadets	7-day camp training	DLW	29	M	3199 ± 453
Margolis et al. (2013)	United States Special Forces	10 days during a 64-day tactics phase	DLW	9	M	5210 ± 717
Margolis, Murphy, et al. (2014)	Norwegian Conscript Soldiers	4-day camp training	DLW	21	M	5480 ± 389
Margolis, Murphy, et al. (2014)	Norwegian Conscript Soldiers	3-day ski march	DLW	21	M	6851 ± 562

Reference	Population	Training Duration & Type	Measurement	*n*	Sex	EE (kcal·d^{-1})
Margolis, Crombie, et al. (2014)	United States Special Operational Forces	7-day pre-mission training	DLW	15	M	3904 ± 521
Margolis, Crombie, et al. (2014)	United States Special Operational Forces	7-day Combat Diver Qualification training	DLW	15	M	4567 ± 350
Fallowfield et al. (2014)	British Royal Marines	7-day deployment in Afghanistan	DLW	18	M	3626 ± 450
Richmond et al. (2014)	British Army Soldiers	10-day training on the Section Commanders Battle Course	DLW	40	M	5091 ± 478
O'Leary et al. (2018)	British Army Recruits	10 days during 14-week basic training	DLW	16	M	4253 ± 556
				17	W	3390 ± 344

2.2 Nutritional Guidelines

2.2.1 *Dietary Reference Values*

Dietary Reference Values (DRVs) are defined as “the amount of food energy needed to balance EE in order to maintain body size, body composition and a level of necessary and desirable physical activity consistent with long-term good health” (Nishida et al., 2007). The requirements for food energy are described for certain population groups, which provide criteria to judge the adequacy of the food intake. Atwater and Woods (1896) published the first formal dietary guidance for workers in the US, with recommendations ranging from 2325 kcal·d^{-1} for a university professor conducting very little exercise, to 8850 kcal·d^{-1} for a brick maker. After the First World War, focus switched from recommended energy to consume, to recommended intake of nutrients, where energy and protein recommendations were set for the British Army by the British Medical Association (BMA, 1950). Developed from the United States National Research Council, the British Medical Association developed a table of recommended nutritional allowances. In 1963, the establishment of the Committee on Medical Aspects of Food Policy (COMA) was developed as an independent source of advice to the government regarding diet and nutritional surveillance (Foster, 2007). In 1969 and 1979, COMA published guidelines which were later updated in 1991 to produce the DRVs, which consisted of a more comprehensive set of values for food energy and nutrient intakes for the UK population (Wiseman, 1992). The report published single figures for macro- and micro-nutrient needs for population sub-groups, which reported a range of calories based on the estimated average requirement for each population group in question (Wiseman, 1992). In March 2000, the committee were disbanded when the Scientific Advisory Committee on Nutrition (SACN) was established to review existing arrangements on nutrition (DH, 2000).

Since the COMA report in 1991, DRVs evolved, as scientific approaches to assessing energy requirements improved. In recent years, the estimated average requirement values for populations have been estimates from DLW-derived measurements of Total Daily Energy Expenditure (TDEE) in reference populations. This approach allowed energy reference values to be framed against those who are more, or less, active than average (25^{th} and 75^{th} percentile) (SACN, 2011). These guidelines were published in 2011 by SACN, increasing the recommended Energy Intake (EI) for men and women by 4% (men: 2500 vs. 2605 kcal·d^{-1} and women: 2000 vs. 2076 kcal·d^{-1}) (SACN, 2011).

2.2.2 *Military Dietary Reference Values*

Based on a collection of the aforementioned guidelines, recommended energy and macronutrient intakes have been suggested for military personnel in the Joint Services Publication (JSP) 456, Defence Catering Manual (2009), to include guidelines for those in training, those in operational settings, and those undertaking non-operational tasks / training. The JSP 456 provides guidance on appropriate energy and macronutrient intake to provide Armed Forces personnel with enough energy to fulfil their military roles, and has formed the basis of the feeding provision that contracted caterers are required to serve within military establishments (Hill et al., 2011). However, these guidelines are regarded as a minimum provision rather than reflecting optimal intake.

More recently, a project tasked by the UK Ministry of Defence's (MOD) Surgeon General was undertaken to investigate the nutritional status of deployed British military personnel and how this might affect body composition, physical fitness and operational capability (Hill et al., 2014). Data from UK military personnel was therefore gathered to inform military dietary recommendations (Table 2.2). Energy expenditure was measured using DLW in Royal Marine recruits and Royal Navy OCs, however although the courses are deemed similar, no data were collected on Army OCs. Furthermore, the dataset collated on military personal was relatively small (8 men and 8 women) and therefore may not be fully representative of the nutritional needs of OCs during training, especially when on exercise.

A more recent report (SACN, 2016), provided updated values based on the data collected for the UK MOD surgeon General (Table 2.2) and the updated physical activity levels from the previous SACN report for each grouping of military personnel. As there was no difference in these values between sexes, men and women were grouped together. Three groups were therefore identified; 1) those in active service 2) those in military training courses such as the military syllabus for recruits and Royal Air Force Phase-1 recruits, and 3) those on military training courses such as the Common Infantry Course, the CC course and the Section Commanders Battle Course, which were further split into percentiles. The median values derived from volunteers from each of these groups represented a reference man (body mass 75.5 kg and height 1.78 m) and woman (body mass 60.0 kg and height 1.65 m). The 25th and 75th percentiles provide guidance with respect to the higher and lower energy requirements, respectively (SACN, 2016). The energy requirements for the median category of active service personnel increased by 24 and 27% for men and women, respectively, from the updated general population guidelines (men: 2900 vs. 3600 kcal·d^{-1} and women: 2200 vs. 2800 kcal·d^{-1}). For OCs during military training, energy requirements, derived from DLW data from 8 men and 8 women on the CC, was increased by 59% for both men and women (as shown in Table 2.3).

Table 2.2: *Studies evaluating Energy Intake (EI) during military training in Men (M) and Women (W)*

Reference	Population	Sample Size	Energy intake (kcal·d^{-1})	Carbohydrate (%)	Protein (%)	Fat (%)	Method
Dziubak et al. (2012)	Royal Navy Recruits (Phase-1)	M 185	2578 ± 851	42 ± 5	17 ± 3	35 ± 4	Food frequency questionnaire
		W 23	2388 ± 793	44 ± 6	17 ± 3	37 ± 4	
Fallowfield et al. (2010)*	Royal Navy OCs	M 89	2725 ± 789	45 ± 7	17 ± 3	38 ± 5	Food dairy
		W 16	2438 ± 335	48 ± 6	15 ± 2	35 ± 4	
Fallowfield, Dziubak, et al. (2012)	Royal Air Force Recruits	M 171	3061 ± 779	Not reported	Not reported	Not reported	Food diary
		W 49	2919 ± 541				
Fallowfield, Shaw, et al. (2012)	Royal Air Force OCs	M 63	2890 ± 541	Not reported	Not reported	Not reported	Food dairy
		W 8	2250 ± 381				

Studies used in the SACN (2016) report to provide evidence for the Surgeon General's Armed Forces Feeding Project which aimed to evaluate the adequacy of feeding provisions in training; * Sample size was based on the number of participants recruited at the beginning. Values were averaged from the start, middle and end of the course.

As there had previously been no direct evidence from military populations about the impact of different macronutrient sources on performance, the UK macronutrient guidelines (for carbohydrate and protein) for military personnel are largely based upon evidence from athletic populations undertaking sports training and competition as well as the recommendations published by the International Olympic Committee Medical Commission (IOC, 2003). Therefore, recommendations are provided (see Table 2.3) as a range for carbohydrate and total fat, with a lower limit set to reflect the recommendations of the general population, with an absolute protein intake remaining constant (SACN, 2016). Individual macronutrient recommendations will be discussed in detail further in Section 2.2.3.

Table 2.3: *Dietary Reference Values (DRVs) of Energy, Carbohydrate (CHO), Protein (PRO) and Fat intake for Men (M) and Women (W) in general, athletic and military populations*

Reference	Guidelines	Population	Sex	Age (y)	Percentile	Recommended Energy Requirements (kcal·d^{-1})	Recommended Macronutrient Requirements (% of daily energy intake)
SACN (2016)	DRVs	Officer Cadets during training	M	-	25th	4400	CHO: 50 - 65%; PRO: 10 - 15%; Fat: 25 - 35%
					50th	4600	
					75th	4900	
			W	-	25th	3400	
					50th	3500	
					75th	3800	
SACN (2016)	DRVs	Active Military Service Personnel	M	-	50th	3600	CHO: 50 - 55%; PRO: 14 - 15%; Fat: 32 - 35%
			W	-	50th	2800	
Potgieter (2013)	Athletic Guidelines	Athletes involved in 2 - 6 hours' exercise, 5 - 6 days per week	-	-	-	2500 - 8000	CHO: 5.0 - 8.0 g·kg·d^{-1}; PRO: 1.4 - 2.0 g·kg·d^{-1}; Fat: 25 - 30%

Reference	Guidelines	Population	Sex	Age (y)	Percentile	Recommended Energy Requirements (kcal·d^{-1})	Recommended Macronutrient Requirements (% of daily energy intake)
SACN (2011)	DRVs	Average civilian adult of a healthy weight and moderately active	M	19 - 24	50th	2772	CHO: 50%; PRO: 0.8 g·kg^{-1}; Fat: N / A
				25 - 34	50th	2749	
			W	19 - 24	50th	2175	
				25 - 34	50th	2175	
SACN (2011)	DRVs	Average civilian adult of a healthy weight and more active	M	19 - 24	75th	3011	CHO: 50%; PRO: 0.8 g·kg·d^{-1}; Fat: N / A
				25 - 34	75th	3011	
			W	19 - 24	75th	2390	
				25 - 34	75th	2390	

2.2.3 *Macronutrients*

Recommended macronutrient intakes for military personnel are shown in Table 2.3 and are set as a percentage of EI for carbohydrate and fat. Due to a lack of evidence to make precise recommendations, a range is provided for carbohydrate and fat intake, depending on the level of activity (a high activity level would require a higher percentage), whereas protein intake remains constant regardless of activity level (SACN 2016). For athletic population, greater amounts of carbohydrate and protein are needed to meet the type, duration and intensity of exercise, and therefore can be more specifically manipulated to meet these demands.

2.2.3.1 Carbohydrate

Carbohydrate is one of the two main energy sources for exercise and its importance for optimal sport performance is well evidenced (Burke & Deakin, 2010; Pochmuller et al., 2016; Rodriguez et al., 2009). Carbohydrate serves as an important energy source, derived from the catabolism of blood borne glucose and muscle glycogen (the stored form of carbohydrate), powering the contractile elements of the muscles, as well as the central nervous system and brain (McArdle et al., 2010). During rest, liver glycogenolysis (the breakdown of glycogen into glucose) maintains normal blood glucose levels, however, during prolonged activity in the fasted state, liver glycogen depletes whilst active muscles continue to catabolise the available blood glucose to continue movement and activity intensity (McArdle et al., 2010). Low blood glucose can cause weakness, hunger, mental confusion and dizziness, impairing exercise and cognitive performance (Jeukendrup, 2010; Kaplan et al.; Schlabach, 1994).

For athletes, providing the muscles with substrates to support their training on a daily basis is critical for optimal adaptation and performance enhancements (Burke et al., 2004; Mata et al., 2019), which is discussed further in Section 2.6. Carbohydrate, in the form of glucose, provides the most immediate source of energy during exercise, as it is oxidised quickly by the body and is readily absorbed (Jeukendrup, 2008); however, body stores are limited (Coyle et al., 1985). Low carbohydrate diets have been shown to impair both high intensity and endurance based performance compared to higher carbohydrate diets, which lead to augmented glycogen stores, translating into a longer time to exhaustion (Baker et al., 2015; Stellingwerff et al., 2011). Conversely, reducing endogenous and / or exogenous carbohydrate availability during short-term (3 - 10 weeks) endurance training can increase mitochondrial enzyme activity and protein content (Morton et al., 2009; Van Proeyen et al., 2011), increasing lipid oxidation (Hulston et al., 2010), which in some instances can improve training capacity

(Cochran et al., 2015). However, this approach during long-term training such as that in the CC, involves potential pitfalls including perturbations to immune function (Nieman, 2007), impaired training intensity (Yeo et al., 2008) and increased muscle protein oxidation .

Carbohydrate requirements are designed to reduce or delay the onset of fatigue, and enhance performance during a single / multiple session(s) of prolonged exercise (Burke et al., 2001). The amount of carbohydrate required varies depending on modality, duration and intensity of exercise (Jeukendrup, 2014). Although current recommendations of carbohydrate for military personnel are set as a percentage of EI (Gillen et al., 2017; SACN, 2016), it has been debated whether carbohydrate should be expressed in absolute amounts or relative to body mass, which is recommended between 3 - 12 g·kg·day^{-1} for athletes (Potgieter, 2013).

These guidelines have been based upon the intensity and duration of exercise, whereby athletes are recommended to consume between 8 - 12 g·kg·d^{-1} for 4 - 5 hours of high-to-extreme intensity exercise, 6 - 10 g·kg·d^{-1} for 1 - 3 hours of moderate to high intensity exercise, and 5 - 7 g·kg·d^{-1} for 1-hour of moderate intensity exercise 5 - 6 days per week (Thomas et al., 2016). However, the type of exercise is also a considerable factor; prolonged endurance exercise leads to muscle glycogen depletion, linking to fatigue and making it difficult to make the energetic requirements of training (Knuiman et al., 2015), compared to resistance exercise that typically reduces muscle glycogen by 24 - 40% (Koopman et al., 2006; Tesch et al., 1986). The International Olympic Committee recommend carbohydrate intake based on activity type, such as endurance athletes undertaking 1 - 3 hours·d^{-1} should consume 6 - 10 g·kg·d^{-1} compared to strength athletes who are recommended to consume 4 - 7 g·kg·d^{-1}. Although the quantity of carbohydrate for strength trained athletes seems low compared to endurance athletes, given the lower relative EE of strength athletes and their requirements for other nutrients, these guidelines alongside strategic time management of carbohydrate intake may be more applicable ensuring carbohydrate availability is optimised at critical time points (Slater & Phillips, 2011). However, these recommendations are based on a wide range and are independent of the type and duration of endurance or strength based activities, as well as the athletic level of the athlete (Jeukendrup, 2014). Therefore recommendations should be fine-tuned with individual consideration of total energy requirements, specific training needs and feedback from training performance (Thomas et al., 2016).

However, despite these recommendations, it has been argued that no rationale has been given for expressing carbohydrate relative to body mass, as there is no clear correlation between body mass and exogenous carbohydrate oxidation (Jeukendrup, 2014). Instead, it is recommended that the advice given to athletes should be in absolute amounts (Jeukendrup, 2014). For military personnel, low absolute carbohydrate intake has been shown to decrease soldier performance on military tasks such as marksmanship (ability to shoot a firearm accurately) (Montain et al., 1997). Therefore a higher carbohydrate intake, whether it be in absolute, or relative amounts, is necessary to enhance recovery, optimise glycogen stores for the next training session (Potgieter, 2013) and enhance / maintain performance when undertaking endurance exercise, high intensity interval training or long duration field exercises (DeBolt et al., 1988).

2.2.3.2 Protein

For protein intake, current UK government guidelines recommend 0.8 $g{\cdot}kg{\cdot}d^{-1}$ for a healthy population (DH, 2000; Rodriguez et al., 2009). There is broad agreement that this intake is insufficient to promote optimal health for active individuals (Phillips et al., 2016). Therefore physically active people, athletes and military personnel, appear to benefit from consuming dietary protein in excess of the RDA (Kreider, 2010; Pasiakos et al., 2015; Pendergast et al., 2011; Potgieter, 2013). More recent guidelines for athletes, suggest an intake of 1.4 - 2.0 $g.kg{\cdot}d^{-1}$ for endurance and resistance trained athletes (Kreider, 2010; Mamerow et al., 2014; Tarnopolsky, 2004). These higher levels of protein intake are known to help attenuate muscle degradation, promote muscle anabolism and spare muscle mass from loss during energy deficits (Areta et al., 2014; Carbone et al., 2013). A protein intake of 3.0 $g{\cdot}kg{\cdot}d^{-1}$ attenuated the decline in time trial performance during an intensified training period compared to an apparently sufficient 1.5 $g{\cdot}kg{\cdot}d^{-1}$ (Witard et al., 2011), suggesting that protein requirements during intensified training may be higher than previously thought (Williamson et al., 2018). However, other studies have shown opposing results where consuming dietary protein two times the RDA failed to provide skeletal muscle protection during a short-term energy deficit, implying that anything above 1.6 $g{\cdot}kg{\cdot}d^{-1}$ may be unnecessary (Pasiakos, Cao, et al., 2013). Further work is required to understand the protein requirements of military personnel.

2.2.3.3 Fat

For athletes and military personnel, fat intake is recommended to be 25 - 35 % of total EI. This intake is important to ensure optimal health, maintain EB and to ensure optimal intake of essential fatty acids and fat soluble vitamins (Potgieter, 2013). High fat intake has been observed previously in US special forces (Tharion et al., 2004), which may occur when EE is high but time to eat is restricted, such as that on field exercise. The higher caloric density of fat compared to carbohydrate or protein during this time may protect against large energy deficits. Although a higher fat diet has been recommended in highly active individuals compared to the general public, excessive consumption of fat is discouraged, due to the concern that it may displace carbohydrate foods and thus prevent adequate carbohydrate intake (Burke et al., 2004).

2.2.4 *Combat Rations*

Combat rations are provided to military personnel during field operations when it is not practical to establish a field kitchen to provide fresh foods (Tassone & Baker, 2017). The rations contain a mixture of packaged food and drink items including main courses, snack items and beverage powders, which when used exclusively, are designed to provide military personnel with appropriate nutrition (Tassone & Baker, 2017).

For the UK Armed Forces, there are currently three different ration scales which are derived from the MDRVs; Standard Rations feeding (Ration Scale-1: 3000 kcal); Arduous Rations feeding, for Phase-1 recruit and OCs training, exercises and operations (Ration Scale-2: 4000 kcal); and Extreme Rations, for those personnel undertaking training or duty activities with extreme energy requirements (Ration Scale-3: 5000 kcal) (Davey et al., 2013). The macronutrient content of the ration packs is listed in Table 2.4; the carbohydrate composition is lower, and fat composition higher, than the MDRVs for Scale-1 and Scale-2. The fat composition of the ration packs is purposely high to offset any time limitations in consuming a larger volume of less energy-dense nutrients (Davey et al., 2013), and to ensure the required energy needs are met. The protein provisions of Scale-1 and Scale-2 ration packs is higher than the MDRVs, reflecting protein requirements of 1.2 - 1.8 $g \cdot kg \cdot d^{-1}$ based on previous reports of individuals undertaking high volumes of physical training (Davey et al., 2013). However, the protein content of the higher energy ration packs is lower, to ensure a higher supply of carbohydrate and fat to fuel arduous exercise.

Although rations are designed to provide appropriate nutrition, military personnel often have insufficient time to prepare meals to eat and 'strip' rations of items they do not want, in order to save weight and space as a result of prioritising combat equipment (Tassone & Baker, 2017). Therefore, ration stripping, coupled with a large TDEE, can result in large energy deficits. The consequences of this practice in military personnel will be discussed further in Section 2.4.3.

Table 2.4: *Energy and macronutrient content of different ration scales*

Ration Scale	Energy (kcal)	Carbohydrate		Protein		Fat	
		Total (g)	% Energy	Total (g)	% Energy	Total (g)	% Energy
Scale-1	3000	344	46	124	16	129	38
Scale-2	4000	495	49	164	15	152	34
Scale-3	5000	625	50	182	13	194	35

2.3 Nutritional Intake in Military Populations

Although nutrition is an integral component of military readiness, the dietary habits of personnel still remains largely unknown. Table 2.5 shows a variety of studies that have investigated the nutritional status of military personnel. All studies, except Margolis, Murphy, et al. (2014), demonstrated low energy and macronutrient intake compared to UK guidelines. Although the personnel in Margolis, Murphy, et al. (2014) exceeded the UK nutritional guidelines, the EE of the conscripts was higher than the energy provided in the extreme rations. Therefore, in order to meet the demands of the exercise, the conscripts would have had to consume all items in the extreme rations, plus additional personal items. It is therefore evident, that despite the guidelines that are in place, military courses such as those described, demonstrate extremely high EE that cannot be solely met with the food and drinks supplied.

Although various studies have investigated the dietary intake of military personnel, only a few studies have been conducted on military personnel in training, particularly in the British Army (Table 2.2). A study by Fallowfield et al. (2011) in Royal Navy OCs during the start, middle and end of their 28-week initial training, showed that the EI of men (men: start 2844 ± 72, middle 2677 ± 669, end 2701 ± 1625 kcal·d^{-1}) and women (women: start 2510 ± 454, middle 2510 ± 526, end 2295 ± 24 kcal·d^{-1}) was below the MDRVs. Macronutrients, reported as a percentage of their EI, were lower than the guidelines for carbohydrate (men: start 44 ± 5, middle 47 ± 6, end 43 ± 9 and women: start 48 ± 5, middle 50 ± 4, end 45 ± 8 %). Women OCs, during the middle of the course, met the guidelines fractionally, however due to the intake being lower than recommended, it would be unlikely that the OCs were reaching an optimal intake of carbohydrate. Protein intake was in line with, and occasionally above, the recommended percentage intake (men: start 16 ± 2, middle 17 ± 2, end 17 ± 4 and women: start 14 ± 2, middle 15 ± 1, end 15 ± 2%), which on average was approximately 111 g for men and 83 g for women. Although the body mass of the participants was not stated in the study, in order to reach the optimal intake of protein recommended by athletic guidelines, the participants would have had to have been 55 - 59 kg for men and 41 - 59 kg for women. Although for women this may have been plausible, albeit slightly low, it is unlikely that the average weight of the men was below 60 kg, especially in the military setting. Therefore, although the protein intake was in line with the MDRVs based on the percentages, relative protein intake was likely still too low. More recently, Chapman et al. (2019) investigated the dietary intake of British Army recruits undergoing Phase-1 training, and demonstrated that the EI of both men and women were less than the MDRVs (men: 2846 ± 573 and women: 2207 ± 585 $kcal \cdot d^{-1}$), as well as demonstrating an under-consumption of carbohydrate (men: 4.8 ± 1.3 and women: 3.8 ± 1.4 $g \cdot kg \cdot d^{-1}$) and protein (men: 1.5 ± 0.3 and women: 1.3 ± 0.3 $g \cdot kg \cdot d^{-1}$). Although EE was not measured, previously estimated EE in Phase-1 training has been reported to be 4253 ± 556 and 3390 ± 344 $kcal \cdot d^{-1}$ in men and women, respectively (O'Leary et al., 2018), therefore deeming it likely that the recruits were in a large energy deficit.

A limitation to most of the studies that evaluate food intake, is that they often rely on participants recalling their dietary intake. Under reporting has been reported within special groups such as the military (Hill & Davies, 2001), therefore low intakes could be an error of misreporting and should be reviewed with caution. Methods for EI are discussed further in Section 3.2.

Table 2.5: *Previously reported dietary Energy Intake (EI) and macronutrient intake of military personnel published in the peer-reviewed literature*

Reference	Population	Participants (n)	Duration	Energy (kcal·d^{-1})	Carbohydrate	Protein	Fat	Method
DeBolt et al. (1988)	US Navy Seals Trainees*	M 267	1-day	3886 ± 73	417 ± 9 g (43%)	151 ± 3 g (16%)	182 ± 5 g (41%)	Food diaries
Bingham et al. (2009)	Finish Conscripts*	M 47	4 days	3401 ± 744	414 ± 105 g (48%)	141 ± 30 g (17%)	127 ± 36 g (34%)	Food diaries
Bingham et al. (2009)	Finish Conscripts**	M 31	3 days	3786 ± 596	478 ± 89 g (51%)	127 ± 22 g (13%)	156 ± 26 g (37%)	Food diaries
Fallowfield et al. (2010)	Navy Officer Recruits*	M 89 W 16	4 days	2725 ± 789 2438 ± 335	45 ± 7% 48 ± 6%	17 ± 3% 15 ± 2%	38 ± 5% 35 ± 4%	Food diaries
Dziubak et al. (2012)	Royal Navy recruits*	M 185 W 23	4 days	2578 ± 851 2388 ± 793	42 ± 5% 44 ± 6%	17 ± 3% 17 ± 3%	35 ± 4% 37 ± 4%	Food diaries
Moran et al. (2012)	Israeli Defence Combat Recruits*	M 74	4 months	2587 ± 879	335 ± 178 g (50%)	114 ± 42 g (18%)	90 ± 32 g (31%)	Food frequency questionnaire
Ramsey et al. (2013)	US military Service Members†	M 18 W 21	12 months	2639 ± 1252 1975 ± 639	290 ± 51 g (44%) 265 ± 92 g (53%)	100 ± 51 g (15%) 87 ± 32 g (18%)	98 ± 53 g (33%) 68 ± 27 g (31%)	Food frequency questionnaire

Reference	Population	Participants (n)	Duration	Energy (kcal·d^{-1})	Carbohydrate	Protein	Fat	Method
Margolis, Murphy, et al. (2014)	Norwegian Conscript training (ration packs)**	M 21	4 days	5480 ± 389	4.4 ± 0.9 g·kg·d^{-1}	1.6 ± 0.3 g·kg·d^{-1}	1.5 ± 0.3 g·kg·d^{-1}	Returned discards & food logs
Fallowfield et al. (2014)	British Royal Marines**	M 80	4 days	2531 ± 798	48 ± 8%	17 ± 3%	34 ± 7%	Food diaries
Royer et al. (2018)	US Special Operation Forces*	M 27	1-day	2752 ± 1073	273 ± 146 g (40%)	155 ± 99 g (23%)	112 ± 51 g (36%)	Dietary recall
Nykanen et al. (2018)	Finish soldiers	M 40	3 days	2414 to 2510	243 ± 78 g (40%)	133 ± 40 g (22%)	96 ± 32 g (35%)	Food diaries
McAdam et al. (2018)	US soldiers, Initial Entry Training	M 111	3 days	2644 ± 639	344 g (52%)	113 g (17%)	88 g (30%)	Diet logs
Chapman et al. (2019)	British Army Phase-1 recruit training	M 17 W 28	8 days	2846 ± 573 2207 ± 585	4.8 ± 1.3 g·kg·d^{-1} 3.8 ± 1.4 g·kg·d^{-1}	1.5 ± 0.3 g·kg·d^{-1} 1.3 ± 0.3 g·kg·d^{-1}	34 ± 3% 41 ± 4%	Dietary food weighing

Men (M), women (W); * Camp Conditions ** Field conditions † Non-Combat environments. Energy and macronutrient data are mean ± SD.

2.4 Energy Balance

2.4.1 *The Concept of Energy Balance*

Energy balance is defined as the balance between EI and EE and is based on the first law of thermodynamics, that energy cannot be destroyed and can only be gained, lost or stored by an organism (Hall et al., 2011).

The basis of EB, is the amount of dietary energy added to, or lost from, the body's energy stores after the physiological systems work has been done, and therefore can be seen as an output from those systems (Loucks et al., 2011). An imbalance can be caused when intake exceeds or is less than expenditure creating a positive or negative balance, respectively, and thus resulting in a gain or loss of body mass. Simply, EB will occur when energy in equals energy out, as shown in Equation 2.1.

$$EB = EI - EE$$

Equation 2.1: *Calculation of Energy Balance (EB)*

The main component of daily energy turnover is the Resting Metabolic Rate (RMR), which represents the amount of energy required to maintain normal body functions and homeostasis (Hill et al., 2013). The second component is the Thermogenic Effects of Food (TEF) which refers to the energy required to absorb, digest and metabolise food consumed, and represents approximately 10% of EE. The final component, Physical Activity EE (PAEE) is the amount of energy expended in addition to RMR and TEF, including involuntary exercise, such as shivering, and voluntary movement including fidgeting and exercising, which accounts for 25 - 35% of TDEE (Hill et al., 2013; Westerterp, 1998). Physical activity EE is as low as 100 kcal·d^{-1} in sedentary individuals and > 3000 kcal·d^{-1} in elite athletes (Hill et al., 2013). Adult humans in western countries generally maintain EB throughout their life, and therefore body mass and body composition show very little fluctuation (Westerterp, 1998).

Energy imbalances are usually episodic in nature (Kurpad et al., 2007), where a change in body mass will occur until EB has reached a new equilibrium (Westerterp, 1998). Within the large-scale Framingham study, body mass measurements of 5209 participants taken every 2 years did not change by more than 10 kg over a 20-year period, and the overall difference between EI and EE was within 1% (Westerterp et al., 1995).

2.4.2 *Consequences of Negative Energy Balance*

A negative EB of ~500 kcal·d^{-1} will result in a 0.5 kg loss of body mass per week (Hall et al., 2011). However, the calculations of energy imbalance and the translation of the imbalance to a change in body mass, is not always straightforward (Hall et al., 2011). It has been established that when EE exceeds EI, the resulting loss in body mass is accounted for by 60 - 80% by Fat Mass (FM) (Hill et al., 2013), and up to 25% Fat Free Mass (FFM) (Hector et al., 2017). The timescale for a steady state change in body mass through an energy imbalance is mathematically given by the effective energy density of the change in body tissue divided by the slope of the relation between the total EE rate and weight change (Chow & Hall, 2008). Both of these factors are influenced by the initial body composition of the individual (Hall, 2010b), thus changes of FM and FFM are related by a non-linear function of the initial body FM (Forbes, 1987; Hall, 2007; Hall, 2010a). As FM contributes less than FFM to overall EE (Hall et al., 2011) a person with higher initial FM will lose a greater amount of body mass to achieve a new state of EB (Hall & Jordan, 2008).

Alterations in EI or EE usually results in some level of reciprocal compensatory change, with the physiological regulation of body mass biased towards maintaining caloric intake and protecting energy stores (Stubbs et al., 2004). The energy content per kilogram of change of FM is 9441 kcal and FFM is 1816 kcal, thus to lose the same mass of fat as lean tissue requires about a fivefold greater deficit in net energy (Hall et al., 2011), and therefore a reduction of EI by the equivocal amount will not lead to a 1 kg decrease in FM / FFM due to the body’s natural sparing effect (Hall et al., 2011). As lean tissue is more energetically expensive to maintain than body fat, it contributes more to the body’s overall EE, and thus energy portioning also contributes to changes in RMR and the energy cost of tissue deposition and turnover (Hall et al., 2011), this is described in detail in Section 2.5.

Based on the aforementioned concept, it is clear why studies investigating EB, do not show a linear decrease / increase in body mass with a negative / positive EB, and therefore the concept proposed by Hall (2008) may explain the differences in the loss of body mass in the studies that investigate EB.

2.4.3 *Effect of Negative Energy Balance on Protein Turnover*

Protein in the human body is in a continuous state of turnover, also known as proteostasis, where new proteins are being made (protein synthesis) and old proteins are being broken down into their constituent amino acids (protein degradation). This cycling of protein synthesis and breakdown is known as protein turnover (Ruddick-Collins et al., 2018) and can provide fundamental information underpinning the relative gains, losses and maintenance of lean body tissue (Waterlow, 2006).

During an acute period of energy deficit, whole body protein turnover is upregulated and proteolysis and oxidation are typically increased to provide amino acids to the working muscles to sustain MPS. However, as the duration of the energy deficit increases, whole body protein turnover, an energy-requiring process, is down-regulated as the body adapts to conserve energy and protein reserves (e.g., muscle protein) (Carbone et al., 2012; Pasiakos, Margolis, et al., 2014). Periods of negative EB can present a major challenge to maintaining muscle mass, as skeletal muscle serves as an amino acid reservoir, and can be readily catabolised to provide precursors for energy metabolism (Carbone et al., 2019). During these times, decrements in the level of Insulin-like Growth Factor-1 (IGF-1) levels (Henning et al., 2011) and the Mammalian Target of Rapamycin (mTOR) signalling (Areta et al., 2014; Pasiakos, Cao, et al., 2013; Pasiakos et al., 2010), co-regulators of muscle hypertrophy, alter muscle mass by decreasing rates of MPS, and increasing Muscle Protein Breakdown (MPB) (Carbone et al., 2012). This promotes an overall decline in net protein balance which can underpin the mechanistic reduction in FFM (Hector et al., 2017). It has also been found that 5' AMP-activated Protein Kinase, a signalling molecule that is initiated during energy depletion in the muscle cells, increases during negative EB and inhibits MPS (Henning et al., 2011). It is also known to be upregulated after repeated muscle eccentric contractions during endurance exercise (Wadley et al., 2006), which is common for military personnel undertaking prolonged load carriage exercises. Subsequently, as the duration of the energy deficit is extended (> 3 weeks), whole-body protein breakdown and synthesis are reduced, conserving energy and endogenous protein reserves (Pasiakos, Margolis, et al., 2014; Stein et al., 1991)

Karl et al. (2016) measured whole-body protein balance in soldiers during 48 hours of a severe energy deficit (-3681 ± 716 kcal·d^{-1}) and during a period of energy balance, in a randomised crossover design. To maintain EB, the amount of EI provided was equivalent to each participants estimated TDEE plus their RMR, whereas the amount of EI provided during the energy deficit was < 10% of that consumed during EB. The energy deficit was induced through increased EE (3947 ± 750 kcal·d^{-1}) and reduced EI (266 ± 61 kcal·d^{-1}). During EB, whole-body protein flux, protein synthesis and protein breakdown were suppressed, while during a two-day energy deficit, protein breakdown increased, with no difference in synthesis and flux; resulting in a more negative net protein balance. These studies demonstrate that acute negative EB may attenuate the activity of key regulatory molecular signalling proteins associated with MPS. However, it seems that the effect of a negative EB on whole-body protein turnover varies depending on the degree and length of the energy deficit period (Pikosky et al., 2008).

2.4.4 *Energy Balance in the Military*

Early studies on human starvation were the first to characterise human performance decrements during an extended energy deficit (Henschel et al., 1954; Keys et al., 1950; Taylor et al., 1957). Since then, a series of studies, such as those conducted by the US Army Research Institute of Environmental Medicine in the early 1990s during Army Ranger training, clearly demonstrate that muscular strength and power output decline after severe weight loss (Friedl, 1995; Nindl et al., 1997; Nindl et al., 2000).

As discussed in Section 2.1, the physical demands of military training often lead to high EE, which are often met with restricted EI (Section 2.3). It is demonstrated that training and combat operations often elicit a more severe energy deficit than those imposed to elicit weight loss in the general public, and a hypo-energetic diet may result in an undesired loss of FFM (Layman et al., 2003; Tharion et al., 2005). Due to the extremity of field exercises, and the training demands in the military, physiological consequences are likely more pronounced than daily training in camp (Hoyt et al., 1991; Hoyt et al., 2006; Margolis et al., 2013; Tharion et al., 2005). In these circumstances, energy deficits, due to constant physical activity, limited access to food and inadequate time or desire to eat, can impact health (Carbuhn et al., 2010), increase injury rates (Dixon & Fricker, 1993; Schlabach, 1994), and compromise physical performance (Friedl, 1995; Montain & Young, 2003).

Although many studies have investigated the EI and EE of military personnel separately, only few studies have explored total energy balance during these periods. Table 2.6 summarises the literature investigating negative EB in military populations, whereby the studies that have investigated EB in military personnel, vary in study length and magnitude of energy deficit. Therefore, despite the understanding of the physiological consequences of a negative EB, it is unknown to what degree and magnitude soldiers can elicit without any harmful effect.

Table 2.6: *Previously reported energy deficits in military personnel published in peer-reviewed literature*

Reference	Population	Participants (n)	Training duration & type	Energy Deficit (kcal·d^{-1})	Method
Margolis et al. (2013)	US soldiers	13 males	10-day simulated urban combat	-2700 ± 540	DLW & dietary observation
Margolis, Murphy, et al. (2014)	Norwegian soldiers	21 males	4-day camp training	-2382 ± 499	DLW & food diaries
Richmond et al. (2014)	British Army soldiers	40 males	8-week Section Commanders' Battle Course	-644 -1343 to -1348	Body mass changes DLW & dietary weighing
Margolis et al. (2016)	Norwegian soldiers	21 males	3-day ski march	-3390 ± 669	DLW & food diaries
Berryman et al. (2017)	US marines	63 males	18-day training school	-4203 ± 1686	Body mass changes
McAdam et al. (2018)	Initial entry US recruits	111 males	7-day camp training	-595 ± 896	Body mass changes

2.4.5 *Effect of Negative Energy Balance on Performance*

Performance decrements in military personnel during periods of negative EB have been mainly attributed to the loss in FFM. Nindl et al. (2007) investigated the physiological consequences of an 8-week military course that involved four different environmental phases and consisted of 7 - 10 days of negative EB (~1000 - 4000 kcal·d^{-1} over the period of 7 - 10 days estimated via menus and rations). From the beginning to the end of the course, performance in maximal lift performance, vertical jump height and peak power output, declined by 20, 16 and 21%, respectively, which was thought to be caused by a decrease in body mass of 12.6% (6% FFM; equating to 6 kg FM and 4 kg FFM) over the data collection period. Another study in Norwegian conscripts found that during a 4-day Military Task Training (MTT), and 3-day Ski March (SKI), a decline in lower body peak power (MTT: -2.8 and SKI: -4.2%) and vertical jump height (MTT: -2.8% and SKI: -6.1%) was reported during a -2382 ± 499 kcal·d^{-1} deficit during MTT and a -3390 ± 669 kcal·d^{-1} deficit during SKI. Interestingly, in this study, body mass only decreased during MTT (-2.2%) despite the greater performance decrease in SKI (Margolis, Murphy, et al., 2014). The differences in performance decrements in these studies may be due to the magnitude and duration of the energy deficit. However, the magnitude of FFM loss or duration and where performance is affected, is not well defined. Field exercises that are short (3 - 7 days) and create small body mass losses (< 3%), are not considered detrimental to performance (Friedl, 1995) compared to longer exercises (8 - 12 days) with greater body mass losses (> 5%) (Pasiakos & Margolis, 2017). The decrease in body mass during military operations of less than three days duration are due to water losses or dehydration rather than loss of FM or FFM (Friedl, 1995). Although Margolis, Murphy, et al. (2014) showed a performance decrement despite a small body mass loss, it is likely that the enhanced stressors such as fatigue, environment and heavy load carriage could have affected performance. A study conducted during a 15-week training course for British Army Infantry soldiers, reported a body mass loss of -5.1 ± 3.1 kg, which accounted for -0.4 ± 1.8 kg FFM and -4.8 ± 3.0 kg FM that was lost within a six-week period (Richmond et al., 2014). Although performance measures were not taken during the study, and despite all participants being in an energy deficit throughout the course, the authors speculated that it was unlikely, due to the little loss of FFM, that there would have been any declines in performance (Richmond et al., 2014).

Interestingly, a meta-analysis by Murphy et al. (2018) showed that decrements in lower body power and strength, following strenuous military training, were not independently associated with daily EB or training duration. However, the combination of daily EB and duration, expressed as total EB (daily EB x duration) was associated with decreases in both lower-body power and strength after military training. Based on the slope equations generated from the meta-regression analysis, the authors reported that a negative EB of -1166 kcal·d^{-1} or a total of -8162 kcal over the duration of the exercise / operation could be endured without any declines in performance. Total negative EB should be limited to -5686 to -19,109 kcal for an entire operation (between 7 to 64 days), for a zero to small effect on performance, respectively. Moderate to large performance decrements would occur with a total negative EB of -39,243 to -59,377 kcal (between 7 and 64 days), resulting in a percentage change in body mass of 7.7% (Murphy et al. (2018).

Military training courses are often designed to purposely induce energy deficit (Nindl et al., 2007; Richmond et al., 2014), through high EE, limited food and sleep. However, a balance is required between the point where military personnel can withstand these conditions, without any harmful consequences to health and performance. Therefore, understanding the magnitude at which negative EB can be tolerated without causing any detrimental harm, would be beneficial. Although energy deficits can be extreme (> 40% of total daily energy needs) in military situations, leading to loss of body mass and decrements in performance, dietary interventions can prevent significant loss of FFM during a negative EB (Berryman et al., 2017; Tassone & Baker, 2017). Increasing protein intake during these periods, can be an effective countermeasure to attenuate protein loss, protecting FFM and decreasing the negative effects associated with negative EB (Areta et al., 2014; Margolis et al., 2016; Mettler et al., 2010). These strategies will be discussed further in Section 2.6.2.2.

2.5 Energy Availability

In nutrition, quantifying EB has historically been the basis for research and practice (Loucks et al., 2011). However, the model of EB assumes that if individuals are in a state of EB that all physiological functions are at an optimal level, which may not be the case (Papageorgiou et al., 2018). It has been proposed that an energy deficient organism may reduce basal metabolism in an attempt to restore EB, albeit with a suppression of non-immediately essential physiological functions (Svendsen et al., 1993; Wade et al., 1996). Myerson et al. (1991), during their research on EB and menstrual dysfunction, found that there was a sparing quality or reduced RMR in some athletes, and that during periods of inadequate metabolic energy, the body may sustain energy consuming activities for survival, whilst less critical energy consuming processes such as the reproductive system or growth may be compromised. This process has now been referred to as EA and has been increasingly researched over the last few years.

The term low EA has more recently been referred to as the mismatch between EI and PAEE or daily work, leaving inadequate energy to support the functions of the body to maintain optimal health and performance (Mountjoy et al., 2018). When EB is disturbed, and EI is less than TDEE, the body's energy reserves (e.g., adipose tissue, body proteins) can contribute to providing energy substrates and / or there is conservation of EE involved with other body functions as an evolutionary adaptation favouring survival (Burke, Lundy, et al., 2018). Evidence for the importance of EA was derived from controlled laboratory investigations which demonstrated that EA is the difference between EI and PAEE, relative to FFM (Loucks, 2003; Loucks et al., 1998), and represents the energy remaining for other metabolic processes to ensure optimal physiological function (Ong & Brownlee, 2017). Energy availability is expressed in Equation 2.2 (Loucks et al., 1998), where FFM is expressed in kilograms, and reflects the body's most metabolically active tissues (Loucks et al., 2011; Mountjoy et al., 2018).

$$EA = \frac{EI - PAEE}{FFM}$$

Equation 2.2: *Calculation of Energy Availability (EA) through Energy Intake (EI), Physical Activity Energy Expenditure (PAEE) and Fat Free Mass (FFM)*

Resting metabolic rate represents the energy cost of basic physiological functions such as immunity and thermoregulation (Fuqua & Rogol, 2013). When EI is inadequate, energy allocation is prioritised to physiological processes essential for survival (Wade & Jones, 2004). Within human evolution, whereby food was either scarce or high in abundance, the energetic priorities would be adjusted for human survival and accordingly some physiological processes that would not be necessary, such as reproduction and fat storage, would be compromised (Wade & Jones, 2004). Due to the similar energy portioning of both these systems, it has led to conclusions that impaired reproductive function is a consequence of low body fat, rather than the fact that they are both direct consequences of low EA (Wade & Jones, 2004). Wade et al. (1996) described how energy demands unaccompanied by compensatory increases in caloric intake, diminished fertility in a vast array of mammalian species, ranging from shrews to whales and including humans. The authors also described how nutritional infertility is particularly common in women, which is not surprising, given that a complete reproductive cycle of ovulation, conception, pregnancy, and lactation is one of the most energetically costly activities that a female mammal will ever undertake, particularly in species that bear multiple young concurrently (Wade et al., 1996).

Low EA can occur through a reduction in EI and / or increased exercise load, causing the body systems to adjust to reduce EE on various physiological functions, preventing further body mass loss (Burke, Lundy, et al., 2018). Low EA (≤ 30 kcal·kg $FFM^{-1}\cdot d^{-1}$) and reduced (30 - 45 kcal·kg $FFM^{-1}\cdot d^{-1}$) EA has been associated with increased risk of impaired physiological functions and / or physical performance (Loucks et al., 2011), increased risk of menstrual disorder and infertility in women (Loucks et al., 2011), reduced testosterone levels in men (Burke, Close, et al., 2018), increased risk of bone stress injuries due to decreased bone resorption in both men and women (Wilson et al., 2015) and increased injury risk (Thein-Nissenbaum et al., 2011). Even further so, low EA has been reported to cause the amount of energy used for thermoregulation, growth, cellular maintenance and reproduction to be reduced by the more crucial physiological mechanisms that are necessary for survival, resulting in health and performance impairments (Mountjoy et al., 2018). Although this compensation seems to reinstate EB, which helps survival, it can damage the long-term health of an individual (Nattiv et al., 2007). Optimal EA for healthy physiological function is therefore reported to be ≥ 45 kcal·kg $FFM^{-1}\cdot d^{-1}$ (Loucks & Thuma, 2003).

Although the reference values for reduced and low EA have been determined for women, it is unknown if the same reference values apply for men. Koehler et al. (2016) demonstrated that a short-term EA of 15 kcal·kg $FFM^{-1}\cdot d^{-1}$ in men supressed leptin and insulin concentrations, but did not impact other metabolic hormones such as insulin-like growth factor-1 and testosterone. The authors of the study concluded that the reference values may well differ from women, and men may be more metabolically robust against short-term energy reductions that their female counterparts (De Souza et al., 2014; Koehler et al., 2016). It is also important to note that although there are specific thresholds at which either physiological functions or performance can be impaired, there is likely to be a large degree of within and between participant variability (Williams et al., 2014). Therefore, it is still inconclusive as to what degree men and women would be affected by low EA.

Although EA is a relatively new concept, and limited studies directly examining EA are available, previous studies investigating metabolic processes, such as RMR, can be used to explore the concept. Stubbs et al. (2004) studied the effect of a negative EB in eight lean men (77 kg, 20% FM) using a calorimeter to measure EE. For seven days, PAEE (840 $kcal \cdot d^{-1}$) through 3 x 40-minute cycling sessions, and EI (2770 $kcal \cdot d^{-1}$) remained constant, resulting in a mean negative EB (1730 $kcal \cdot d^{-1}$). Interestingly, the constant low EA each day resulted in compensatory daily reductions in TDEE by -76 $kcal \cdot d^{-1}$, which were projected to restore EB to zero within three weeks due to the suppression of physiological systems. Thus, although projected to be at stable EB after three weeks, physiological functions are shown to be compromised which may therefore hinder performance and health (Koehler et al., 2016; Wilson et al., 2015). This adaptation has also been shown by Friedlander et al. (2005), who investigated the response of healthy men during a 40% calorie deficit for 21 days. Although EE and PAEE was not described in the paper, the authors illustrated that the participants lost 3.8 ± 0.3 kg (~5%) body mass over the period, and Basal Metabolic Rate (BMR), measured via indirect calorimetry, decreased by 15% over the intervention. These data provide a useful illustration of how EB can be achieved over time in an energy-deficient state, through suppression of physiological and metabolic processes (Loucks et al., 2011).

Due to this concept, in military settings, a stable body weight, especially during times of arduous training, may not be an appropriate indicator of optimal physiological functions. Margolis, Murphy, et al. (2014) found that Norwegian soldiers lost no body mass during a three day arctic military exercise despite expending up to 6800 $kcal \cdot d^{-1}$ (measured via DLW) and eating approximately 3400 $kcal \cdot d^{-1}$ (measured via dietary weighing), equating to 50% of the energy required to maintain EB. Although the authors did not report EA, it could be calculated (using Equation 2.1), based on an average FM of an active male, that the soldiers were possibly in an extremely low EA. Therefore, although studies such as this have reported no body mass loss, it is unknown whether any physiological, metabolic, and functional systems were compromised in response to the low EA.

Menstrual cycle disturbances associated with energy deficit are dose dependent, with greater energy deficits leading to more frequent menstrual disturbances (Williams et al., 2015). Although women were previously considered at greater risk of low EA (Loucks et al., 2011), more recent reviews of the literature have confirmed that their male counterparts are also at risk, although this may involve different physiological issues to those seen in women (Mountjoy et al., 2014). Low EA has been observed in weight-sensitive sports, where leanness and / or body mass are important factors for performance (long-distance running, road cycling, boxing, wrestling etc.) (Sundgot-Borgen et al., 2013). In such scenarios, reductions in RMR, testosterone levels (Burke, Lundy, et al., 2018; Hackney, 2020) and bone mineral density (Barry & Kohrt, 2008) have been reported. One study in jockeys, who have been shown to have dangerously low EI during a competitive season, observed impaired hormonal markers, such as testosterone levels, compared with controls (Dolan et al., 2012). Therefore, it seems plausible that men and women may be equally susceptible but the consequences seem to be greater in women given the higher essential fat requirements. Weight class sports often implement strategies to 'make weight', by manipulating body composition via techniques such as fluid restriction or fasting (Reale et al., 2017; Smith et al., 2001). In these scenarios, acute weight loss can exceed 10% of total body mass within 24 hours (Alderman et al., 2004; Crighton et al., 2016) resulting in periods of EA as low as < 5 kcal·kg FFM^{-1}·day^{-1}. Despite the increase in awareness and interest in EA, little is currently known currently about the time-frame and magnitude in which low EA can have a detrimental effect. One study demonstrated a decrease in testosterone and serum leptin in French soldiers when in a large energy deficit (-1800 kcal·d^{-1}) during a 5-day combat course (Gomez-Merino et al., 2002). However, in contrast, Gifford et al. (2019) demonstrated no change in resting or sleeping RMR measured as a result of a 61-day 1700 km sled pull across the artic, whilst in a large energy deficit (indicated by body mass loss; 9.37 ± 2.31 kg).

2.6 Feeding Strategies

Previous research has demonstrated that the timing of dietary intake may significantly affect the adaptive response to exercise (Kerksick et al., 2008). This section will discuss feeding strategies for military personnel undertaking strenuous training, multiple times per day, and over prolonged periods, in order to meet the daily requirements to achieve EB and EA (Rodriguez et al., 2009) whilst maximising training adaptations and optimising recovery between exercise sessions (Jeukendrup, 2017).

2.6.1 *Carbohydrate*

At the onset of exercise, glucose supplies the predominant source of energy. Throughout the duration of exercise, both muscle and liver glycogen are broken down through glycolysis to provide an energy substrate to active tissues; liver glycogen is broken down to glucose and released into the blood stream where it is available for all tissues, whereas muscle glycogen is immediately available to the working muscle in which it is stored (Maughan & Gleeson, 2010). During moderate prolonged physical activity, the liver and muscle glycogen supply 40 - 50% of the energy requirement with the remainder provided by fat catabolism and a limited amount of protein (McArdle et al., 2010), which can vary depending on the relative energy intensity. As the body stores of carbohydrate are limited (Coyle et al., 1985), during moderate high intensity exercise (65 - 85% $\dot{V}O_{2max}$), glycogen stores will last a few hours (Tarnopolsky et al., 2005), and as glycogen levels diminish, exercise intensity and work output will decrease (Coyle et al., 1985). Therefore, sports nutrition guidelines promote a variety of options for acutely increasing glucose availability for an exercise session, whilst sparing muscle and liver glycogen in order to delay the onset of fatigue and enhance exercise capacity and performance (Burke et al., 2011).

2.6.1.1 Timing of Carbohydrate Intake

Manipulating carbohydrate before and during exercise, and in the recovery periods between prolonged exercise bouts, provides a variety of options to maximise endogenous glycogen stores and to maintain glycogen levels during exercise (Kerksick et al., 2008), increasing exercise capacity and improving exercise performance (Jeukendrup, 2008, 2010), as well as delaying the onset of fatigue by 10 - 20% in endurance events lasting more than 90 minutes (Coyle et al., 1986). Training in a low glycogen state has recently become a popular strategy to enhance metabolic adaptations to a given training stimulus, with the increased ability to utilise fat as an exercise source with a reduced resilience on carbohydrate (Burke et al., 2011). Although training on a low carbohydrate diet is associated with an increased ability to oxidise fat during exercise and a decreased reliance on muscle glycogen utilisation, there are also indications that it can reduce the chronic adaptations to training and impair carbohydrate utilisation (Stellingwerff et al., 2006) and the ability to sustain high-intensity exercise performance (Havemann et al., 2006). Therefore, whilst restricting carbohydrate around training may be beneficial for training adaptations in athletes before a competition, for military personnel the negative implications are more likely to outweigh the benefits.

Guidelines for ingestion of carbohydrate during exercise are dependent upon the duration of the exercise bout, and generally increase linearly with duration (Jeukendrup, 2014), such that 30 - 60 g or 0.7 $g \cdot kg^{-1}$ of carbohydrate is needed for every hour of exercise (over 1-hour of initial exercise) (Rodriguez et al., 2009). A previous investigation on the effect of 32 - 42 g carbohydrate every hour during 10 hours of discontinuous exercise (50 minutes of exercise every hour), resulted in a 52% higher net muscle glycogen use in the placebo trial, compared to those taking carbohydrate (Harger-

Domitrovich et al., 2007). In the context of military activity, the provision of carbohydrate during exercise may allow for a greater carbohydrate utilisation, and thus increasing intensity and performance.

Post-exercise, muscle glycogen is typically restored to pre-exercise concentrations within 24 hours, providing a sufficient amount of carbohydrate is ingested (Beelen et al., 2010). During the first 30 - 60 minutes following exercise, muscle glycogen restoration rates are high. However, if ingestion of carbohydrate is delayed several hours, the rate of synthesis can decrease by 60 - 90% (Price et al., 1994). The increased synthesis immediately post-exercise is due in part to faster rate of muscle glucose uptake as a result of an increase in muscle insulin sensitivity (Ivy et al., 2003), and an increase in the concentration of glucose transporters within the plasma membrane of the muscle (Ivy, 1998; Ivy & Kuo, 1998). Ivy (1998) reported that an intake of > 0.5 $g{\cdot}kg{\cdot}h^{-1}$ is necessary to maximise post-exercise glycogen synthesis if carbohydrate is administered at 2-hour intervals, however, higher glycogen synthesis rates have been reported in studies in which carbohydrates were ingested more frequently (Doyle et al., 1993; Van Hall et al., 1998). Although post-exercise carbohydrate feeding is clearly beneficial for restoring muscle glycogen, post-exercise feeding may not be possible for military personnel due to time restrictions during training. A study by Berryman et al. (2017) investigated the use of carbohydrate supplementation after a period of severe energy deficit to restore FFM and protein balance during a refeed period. Participants in the control group (high in carbohydrate) received 64 g carbohydrate in the day and 113 g in the evening, compared to the moderate and high protein group who received 32 g and 36 g of carbohydrate in the day, and 56 g and 68 g of carbohydrate at night, respectively. Supplementation of carbohydrate and protein showed no difference in restoration of net protein balance nor FFM. However, as participants were also able to eat *ad libitum*, it can be seen in the results of the study, that overall diet composition did not differ between groups despite having a higher intake of carbohydrate or protein. Regardless of group, all participants ate 5.9 - 6.6 $g{\cdot}kg{\cdot}d^{-1}$ of carbohydrate and 2.4 - 2.7 $g{\cdot}kg{\cdot}d^{-1}$ of protein. Therefore, it may be recommended that increasing EI regardless of macronutrient is more important before, during and after intense exercise periods to restore negative EB than individual macronutrients.

2.6.1.2 Effect of Carbohydrate on Performance in the Military

For soldiers, carbohydrate serves as the main energy substrate for physical military training and together with adequate intake they are essential for recovery (Beals et al., 2015). As mentioned previously, during an energy deficit, essential metabolic alterations are triggered to mobilise endogenous lipids and preserve endogenous carbohydrate, thus suppressing carbohydrate oxidation (Horowitz et al., 2005).

Repeated high-intensity tasks often included in military training indicate a heavy reliance on carbohydrate as an energy source. Suboptimal intake could result in premature muscle glycogen depletion during training, decreased power output and early onset fatigue (Beals et al., 2015). Previous studies examining the effect of carbohydrate intake on rifle shooting performance, before, and after, a loaded march demonstrate that shooting accuracy was significantly impaired when on a low carbohydrate (250 $g \cdot d^{-1}$) diet, compared to a moderate (400 $g \cdot d^{-1}$) or high (550 $g \cdot d^{-1}$) carbohydrate diet (Tharion & Moore, 1993). Blacker, Williams, et al. (2010) investigated the effects of a carbohydrate and a protein supplement, twice daily for three days, on recovery of muscle function after a two hour loaded march, and found that both carbohydrate and protein supplements resulted in faster recovery of neuromuscular function compared to a placebo (water). Although carbohydrate after exercise has been shown to improve muscle glycogen synthesis (Millard-Stafford et al., 2008), the intake of carbohydrate in the aforementioned study was low, and therefore was not considered optimal for restoration of muscle glycogen. Therefore, the positive effect on the recovery of muscle function was possibly due to the higher insulin response, promoted by the carbohydrate supplement, through increasing the rate of MPS at rest and attenuating the rate of MPB after exercise (Blacker, Williams, et al., 2010). The authors speculated that the carbohydrate beverage after exercise, compared to a placebo, decreased the negative protein balance, slowing down the degradation of structural proteins with a positive effect on recovery of muscle function.

Additionally, carbohydrate supplementation has been demonstrated to mitigate inflammation that may be exacerbated during strenuous exercise such as load carriage (Pasiakos et al., 2016). Research in US soldiers investigated the effect of a high carbohydrate supplement (1058 kcal, 189 g carbohydrate, 11 g protein and 29 g fat) compared to a high protein supplement (1062 kcal, 102 g carbohydrate, 85 g protein and 35 g fat) on inflammatory markers during a four-day ski march where participants were in a -3200 $kcal \cdot d^{-1}$ deficit (Pasiakos et al., 2016). The authors reported that hepcidin, a protein associated with inflammation during intense physical exercise and can be exacerbated by depleted energy stores, was increased post-exercise in the control group (no supplement) relative to both supplement (high carbohydrate vs. high protein) groups. However, despite the results, negative EB explained a significant proportion of the results, which suggested that additional energy in the form of both carbohydrate and / or protein, might attenuate declines in hepcidin that result from repeated exposure to intense activity. The results highlight the importance of increasing overall intake during times of energy deficits.

It is difficult to provide definitive guidelines for carbohydrate intake in relation to the requirements of specific training sessions, due to the metabolic demands of military training (in terms of both total EE and carbohydrate needs) (Burke et al., 2011). The most recent guidelines for carbohydrate intake for training recognise the need for flexibility and individual differences among athletes (Bartlett et al., 2015). Although the evidence from athletes may be useful in understanding optimal carbohydrate strategies, it must be acknowledged that in the military setting, where multiple types of activities are performed at varied intensities and durations, as well as often training multiple times per day, no practical feeding strategy for carbohydrate has been recommended. Therefore, there is a need to understand the energy requirements and the nutritional timing of carbohydrate around exercise in order to recommend practical feeding strategies to maintain optimal recovery and performance.

2.6.2 *Protein*

For an active population, recommended guidelines for protein intake were described in Section 2.2.3.2. Although classed as 'tactical athletes', the protein recommendations for military personnel remains at 10 - 15% of total intake, which equates to approximately 0.8 $g \cdot kg \cdot d^{-1}$ for an average male. However as stated previously, after consideration that soldiers often fail to consume adequate energy to match their energy demands (Margolis et al., 2013; Nindl et al., 2007; Tharion et al., 2005), a recent consensus was developed in the United States that recommended soldiers consume 1.5 - 2.0 $g \cdot kg \cdot d^{-1}$ of protein (Margolis, Crombie, et al., 2014; Pasiakos, Montain, et al., 2013). The increased intake was aimed to mitigate declines in whole-body protein turnover during times of high metabolic demands (Beals et al., 2015), protect FFM (Calbet et al., 2017) and improve performance (Moore et al., 2014; Phillips & Van Loon, 2011). Although the total intake is key, the timing of intake can also play an important role within the diet.

2.6.2.1 Timing of Protein Intake

Considerable work has been undertaken to determine the optimal timing of nutritional intake in order to maximise post-exercise MPS and ensure adaptations to training (Atherton & Smith, 2012; Cribb et al., 2006). Pioneering work discovered that exercise and macronutrient feeding interact synergistically to provide a net anabolic effect far greater than feeding or exercise alone (Biolo et al., 1997; Rennie, 2001). Further studies later showed that an acute feeding of amino acids increased rates of MPS (Tipton et al., 1999), which was later shown to be increased for up to 24 hours after feeding (Burd et al., 2011). These studies provided the basis of the theoretical concept of protein timing for resistance and endurance exercise (Breen et al., 2011). While earlier investigations reported positive effects from consumption of amino acids (Biolo et al., 1997; Elliot et al., 2006; Rennie, 2001), it is now clear that intact protein supplements can evoke an anabolic response that can be similar or greater in magnitude to free form amino acids, assuming ingestion of equal essential amino acid amounts (Farnfield et al., 2012).

After exercise, dietary amino acids are required to replace endogenous oxidative losses and to provide building blocks to repair and remodel body tissues (including muscle) during recovery (Williamson et al., 2018). It is now known that MPS rates peak within three hours and remain elevated for an addition 24 - 72 hours after exercise (Burd et al., 2011; Miller et al., 2005). This stimulation of MPS is also combined with peak elevation in signalling proteins, pivotal for increasing the initiation of translation of muscle proteins (Jager et al., 2017). Results from previous studies have found that timing of protein two hours before or after aerobic and anaerobic exercise appears to provide a greater activation of the molecular signalling pathways that regulate myofibrillar and mitochondrial protein synthesis, as well as glycogen synthesis (Coffey et al., 2011; Ivy et al., 2008). Although the majority of studies have investigated the enhanced effect of MPS immediately following exercise, few studies have investigated the effect on MPS within the recovery period 48 hours following exercise. Moore et al. (2009) investigated the protein dose response of MPS up to 4 hours after resistance training in healthy subjects, and found that MPS was maximally stimulated after 20 g of whole egg protein compared to 0, 5, 20 or 40 g. Although no changes were seen in the phosphorylation of the intracellular signalling proteins by ingestion, Moore et al. (2009) state that the resistance exercise in itself, may have possibly stimulated the phosphorylation of proteins within the mTOR-signalling pathway, thereby masking any amino acid induced changes with protein ingestion. Equally, Macnaughton et al. (2016) demonstrated that when resistance trained men ingested whey-protein after a one-hour whole-body resistance training session, 40 g of protein stimulated MPS by 20% more compared to 20 g. Interestingly, a greater amount of FFM did not affect MPS in both groups and thus seems a less important determinate for the optimal dose of protein. The difference in results seen by these two studies may be due to the methodological differences, such as the first used leg only resistance exercises, and the latter used whole-body exercises where the overall demand of amino acids would have been higher due to the greater amount of muscles being activated (Macnaughton et al., 2016). Similarly, Areta et al. (2013), investigated the timing of protein and the rate of MPS when multiple doses were consumed over 12 hours, rather than one dose over six hours previously described by Moore et al. (2009). The study investigated the effect of three different patterns of digesting 80 g of protein during a 12-hour period. The results showed that when 20 g of protein was consumed every three hours for 12 hours after a leg-based exercise bout, MPS was greater compared to 40 g every six hours or 10 g every 1.5 hours, with great implications for protein feeding later in recovery (> 4 hours' post-exercise). However, although the study demonstrated promising results, the total protein supplied to the participants after the single bout of exercise was less than 1 $g \cdot kg^{-1}$, therefore a higher dose may be more beneficial, especially during whole-body exercise and with multiple training bouts per day, as per military training.

Overnight sleep is typically the longest post-absorptive period during the day, and MPS rates have been shown to be surprisingly low during this time (Beelen et al., 2008), which is speculated to be due to limited endogenous availability of amino acids from the gut and / or the intramuscular free amino acid pool (Res et al., 2012; Trommelen & van Loon, 2016). Protein intake immediately prior to sleep has been found to be effectively digested and absorbed, thereby increasing amino acid availability, stimulating MPS rates, and thus improving whole-body protein net balance during overnight recovery (Trommelen & van Loon, 2016). Res et al. (2012) evaluated the effect of protein ingestion on MPS immediately after exercise during subsequent overnight recovery. The authors reported that when 20 g of protein (with an additional 60 g of carbohydrate) at 21:00 hrs (4 hours before sleep), was consumed, it showed effective digestibility and absorption, increasing overnight plasma amino acid availability and stimulated MPS, thereby improving overnight protein balance.

Although most of the studies investigating timing of protein intake are on athletes, little has been investigated in physically demanding occupations such as the military, where time / opportunity to eat may be more limited. As suggested by Areta et al. (2014), 80 g of protein split into four small meals was superior for stimulating MPS, however in settings such as field exercises, eating this frequently may be unlikely. Similarly, Mamerow et al. (2014) found that consuming moderate amount (30 g) of high quality protein three times per day stimulated 24-hour MPS by 25% than the common practice of skewing protein consumption towards the evening meal. Therefore, in the military setting when time to eat is limited, it would be important to consider an equal distribution over the three core meals.

Although studies have investigated post-exercise protein digestion due to the stimulation of MPS after exercise, other studies have suggested that pre-exercise and intra-exercise feeding may present favourable changes in protein balance (Beelen et al., 2011; Bird et al., 2006a; Tipton et al., 2001). Both Fujita et al. (2009) and Tipton et al. (2004) found that ingestion of protein pre-exercise elevated rates of MPS similar to that seen after exercise. However, given the vast amount of studies on protein digestion surrounding exercise, all within different populations, at different timings, and using different training programs of varying intensity's and durations, it is clear that there is no optimal intake of protein surrounding exercise, and thus total intake per kilogram of body mass combined with an equal distribution throughout the day should be considered.

2.6.2.2 Protein Supplementation during an Energy Deficit

As previously described in Section 2.4.2, whole-body protein turnover is essential for uptake and maintenance of lean tissue (Ruddick-Collins, 2017). Turnover rates of approximately 300 g of protein occurs on a daily basis in human adults (~4 $g{\cdot}kg{\cdot}d^{-1}$) equating to 3 - 4 times an individual's daily protein intake. The maintenance of skeletal muscle mass is therefore dependent on the balance between the fasted and fed state, and changes in rates of MPS and MPB (Hector et al., 2017). During energy restriction, MPS is reduced in both the fasted and fed states, promoting an overall decline in net protein balance and causing a reduction in FFM (Hector et al., 2017). Therefore, consuming dietary protein at levels exceeding the recommended dietary allowance during negative EB, has been shown to upregulate whole-body protein turnover, improving net protein utilization (Pasiakos, Austin, et al., 2013) and minimising the loss of muscle protein (Carbone et al., 2012; Longland et al., 2016), which is crucial in maintaining military performance (Henning et al., 2011; Longland et al., 2016) and decreasing susceptibility to injury (Pasiakos, Cao, et al., 2013; Tharion et al., 2005). However, the extent to which these metabolic processes are affected depends largely upon the degree in which energy is restricted (Pasiakos et al., 2010). An investigation by Pikosky et al. (2008), examined nitrogen balance and protein turnover over 7 days when consuming a high protein (1.9 $g{\cdot}kg{\cdot}d^{-1}$) or moderate protein (0.9 $g{\cdot}kg{\cdot}d^{-1}$) diet on a -1000 $kcal{\cdot}d^{-1}$ deficit, induced from increased EE from exercise alone, compared to an energy balanced control period. The study found that the high protein diet prevented a decrease in nitrogen balance compared to the energy balance period, whereas no difference was found between protein groups for protein synthesis, protein breakdown or net protein oxidation during either the EB or negative EB period. The results indicated that the higher protein diet acted to preserve nitrogen balance during the negative EB period, and therefore would be beneficial in populations where short periods of a negative EB often occurs.

Other studies (Pasiakos, Cao, et al., 2013) have investigated whole-body protein turnover in weight stable adults in response to either the RDA (0.8 $g{\cdot}kg{\cdot}d^{-1}$), two times the RDA (1.6 $g{\cdot}kg{\cdot}d^{-1}$), or three times the RDA (2.4 $g{\cdot}kg{\cdot}d^{-1}$) during a 21-day energy deficit of 40%. This study showed that consuming protein beyond the RDA did not elicit any further influence on MPS, and an energy deficit attenuated whole-body protein turnover independent of dietary protein level, as net turnover, whole-body protein synthesis and breakdown were lower in the energy deficit state. In regards to body composition, despite loss in FM, there was no difference between groups (-1.7%). However, in proportion of total weight lost, the group consuming two times the RDA had a greater decrease in FM whilst sparing FFM compared to the other two groups. These results therefore demonstrate that consuming protein greater than the RDA may be important during times of short-term negative EB, however no difference was found when consuming three times the RDA.

In contrast to Pasiakos, Cao, et al. (2013), Mettler et al. (2010), found that consuming a greater amount of protein (2.3 $g{\cdot}kg{\cdot}d^{-1}$), similar to the three times RDA group in the latter study was superior in sparing FFM during a 40% energy deficit compared to 1.0 $g{\cdot}kg{\cdot}d^{-1}$. However, the authors only compared the high and low intake of protein, and therefore along with the results from Pasiakos, Cao, et al. (2013), it may be suggested that a plateau exists, above which consuming dietary protein at greater than two times the RDA confers no additional benefit in the context of short-term weight loss. Both studies required participants to partake in exercise during the energy deficit period, however the latter required resistance type exercise and the former, endurance-based exercise. Due to the metabolic adaptations to the various modalities of exercise, it may well explain the difference in results.

Contradictory to these previously reported findings, Alemany et al. (2008) investigated the hormonal and body composition responses of US Marine Infantry Officers, whilst consuming low protein diets (0.5 vs. 0.9 $g{\cdot}kg{\cdot}d^{-1}$) in a -2400 $kcal{\cdot}d^{-1}$ energy deficit over, an 8-day period. The results showed that body mass declined by -3.2 kg (FFM -1.2 kg) independent of diet group. Although there was a subtle attenuation in hormonal markers such as insulin-like growth factor-1, these were not affected by the level of dietary protein intake. Although the results indicate that the protein intake may have been too low, it may be that the negative EB exceeded a threshold, whereby protein intake could have attenuated any negative effects.

Areta et al. (2014) investigated the effect of protein feeding on resistance trained participants in an energy deficit of 33% for five days. The participants consumed either 15 g and 30 g, equating to 0.2 or 0.4 $g{\cdot}kg{\cdot}d^{-1}$, respectively, in addition to their *ad libitum* diet of 1.4 to 1.6 $g{\cdot}kg{\cdot}d^{-1}$. Although MPS was reduced in the energy deficient state, resistance exercise in combination with the protein intake, increased protein availability, and thus MPS post-exercise, by ~34% above resting level in the higher protein group compared to ~16% in the lower protein group. This was shown to lead to preservation of FFM and was evident in both terms of absolute values and relative to body mass and FFM.

Taken together, these studies highlight the catabolic nature of negative EB and the associated muscle sparing effects of consuming a high protein diet. Regardless of whether an energy deficit is induced by energy restriction, increased EE or a combination, varied measures of whole-body and skeletal muscle protein metabolism indicate that consuming dietary protein in excess of the RDA confers a level of protection for skeletal muscle integrity (Carbone et al., 2012).

Within most studies that investigate the effect of a dietary protein supplement to maintain whole-body protein turnover, the dependant variables (EI, EE and thus EB) are usually highly controlled in a laboratory setting. In the military environment, there is a large day-to-day variation in EB and *ad libitum* eating behaviours. Studies investigating whole-body protein turnover in the field environment, have found that a four per day carbohydrate or protein supplement can spare protein turnover and FFM when in a sever energy deficit (-54%) (Margolis et al., 2016). The results demonstrated that regardless of macronutrient source, weight loss was the same, and it was a result of the increased EI from the supplements (42% of total intake) that attenuated the negative EB and spared whole-body protein. However, this was only seen in those who consumed enough energy to elicit a moderate deficit (~40%). The data also showed that 93% of the participants who maintained a protein balance were in the protein group, suggesting that a protein supplementation is beneficial to achieve a more manageable energy deficit, and possibly positively impacting skeletal muscle mass and performance.

Similarly, Fortes et al. (2011) investigated the effect of a mixed nutritional supplement (~45% carbohydrate, ~15% protein and ~40% fat) on body composition changes and physical performance on soldiers whilst in a negative EB during an arduous field exercise. Although EB was not directly investigated in the study, previous work on the same course found that participants were in a negative EB of 645 kcal·d^{-1} over the 8-week course calculated through changes in body mass (-5.1 ± 3.1 kg) (Richmond et al., 2010). Fortes et al. (2011) showed that over the 8-week training course, the control group, that received no supplement, lost a greater amount of body mass (5.0 ± 2.3 kg) compared to the supplement group (1.6 ± 1.5 kg), with the control group also having greater reductions in FFM (control group: 2.0 ± 1.5 vs. supplement group: 0.7 ± 1.5 kg). The control group experienced greater decrements in vertical jump height and explosive leg power, calculated from jump height and body mass, compared to the supplement group. As the overall daily intake of protein was not measured, the results cannot determine whether the protein within the supplement was responsible for the positive changes or whether the overall increase in calories blunted any negative outcomes caused by an energy deficit. It is clear that within the military field setting, increasing calorific intake regardless of macronutrient source is essential to mitigate negative effects of negative EB. However, consuming protein either alone or in a mixed macronutrient meal or supplement can promote an increase in MPS (Pasiakos et al., 2015), due to the anabolic response to protein ingestion occurring as a result of postprandial increases in extracellular amino acid availability, and thus will aid in maintaining whole-body protein turnover and FFM.

2.6.3 *Benefit of a Mixed Macronutrient Supplement*

As discussed in sections 2.6.1 and 2.6.2, both carbohydrate and protein have their own distinct functions, yet both work simultaneously to generate an anabolic state within the body (Poole, 2010). Although dietary protein intake is required to reach a positive protein balance and to increase MPS, the addition of carbohydrate to protein has been shown to further elevate plasma insulin levels (Van Loon et al., 2000). This has been speculated to stimulate the uptake of selected amino acids and MPS rates (Koopman et al., 2005). Mixed macronutrient supplementation during heavy periods of exercise has been suggested to result in markedly different global transcriptional response compared to consuming carbohydrate or protein alone (D'Lugos et al., 2016), and that a meal containing carbohydrate and protein provided post-exercise would stimulate messenger ribonucleic acid translation and initiation of MPS at a greater rate (Ivy 2004). Protein breakdown has also been shown to be reduced after exercise with consumption of a carbohydrate supplement, resulting in a greater protein balance (Børsheim et al., 2004), indicating that carbohydrate improves overall protein balance when ingested after exercise.

Similarly, the addition of protein to carbohydrate supplements, has been shown to increase the rate of glycogen storage post-exercise compared to a carbohydrate supplement or calorific equivalent treatment (Ivy et al., 2002). This is believed to be due to the greater insulin response after ingestion of a carbohydrate-protein mix, which can lead to an increase in glycogen synthase activity (Van Loon et al., 2000). Insulin stimulates glucose utilisation by muscle cells through activation of glucose transporters and stimulation of intracellular enzyme pathways for oxidative and non-oxidative glucose metabolism (Ivy, 1998; Ivy & Kuo, 1998). Van Loon et al. (2000) found that the plasma insulin response to consumption of a carbohydrate-protein supplement was 88% greater than consumption of a carbohydrate supplement alone. The authors also found that increasing carbohydrate intake to a similar calorific value created similar results, indicating that a mixed macronutrient supplement can be beneficial, but glycogen synthesis rates can also be accelerated by increasing carbohydrate alone.

Other additional benefits of a combined macronutrient supplement have been shown, whereby muscle function is better preserved after heavy endurance exercise, and indices of post-exercise muscle damage are reduced compared to consuming carbohydrate alone (Berardi et al., 2008; D'Lugos et al., 2016; Greer et al., 2007). However, other studies have demonstrated no benefit in performance (Berardi et al., 2006) or recovery (Gilson et al., 2010) with the intake of mixed carbohydrate and protein supplements. Therefore, although there has been mixed observations as to whether a single or mixed macronutrient supplement provide benefits during negative EB, it still remains clear that feeding strategies that attenuate EB by increasing EI during times of large energy deficits, can be beneficial.

2.6.4 *Fat*

Although studies investigating feeding strategies have focused on carbohydrate and protein, only few studies have focused on dietary fat as a supplement. The recommended intake of fat is based on the need to ingest adequate amounts of essential fatty acids (Horvath et al., 2000). In light of the low carbohydrate diet to utilise fat as an energy source with a reduced resilience on carbohydrate, some researchers have suggested an increased fat availability over 24 hours, can increase muscle triglyceride stores and can enhance endurance exercise performance by delaying the onset of carbohydrate depletion and fatigue (Starling et al., 1997). It has also been speculated that if fat intake increased whilst maintaining sufficient carbohydrate intake, it is possible that endurance exercise time could even be improved (Pendergast et al., 2000).

Given low fat diets, implemented by athletes, reduce carbohydrate utilisation, a study by Horvath et al. (2000) examined changes in energy, vitamin and mineral intake with different levels of dietary fat (low 17%, medium 31% and high 44%) for 28 to 31 days in runners. The results showed that protein was still within the recommended range, and carbohydrate only differed a small amount, therefore total caloric intake was better matched to activity EE when on the higher fat diets compared to the low fat diet, and despite the higher caloric diet from fat, body weight and body fat percentage remained unchanged. The results of the study indicate that individuals with high daily EE, such as the military, may benefit from a higher fat intake in order to increase EI, improve metabolic pathways that use fatty acids and help in the utilisation of nutrients that are absorbed or transported with fat (Horvath et al., 2000).

2.7 Literature Review Summary

Military training is often physically and cognitively demanding and can often lead to times of negative EB. Nutritional intake has been shown to affect physical and cognitive performance of personnel and influence their recovery from- and adaptation to- training. Optimising nutrition in the military setting is difficult due to the variation in the demands of training within and between days, particularly when personnel are moving between camp, field exercise and operational settings. Therefore, ensuring personnel consume adequate nutritional intake to match their physical activity patterns is critical in the military setting.

The research presented in this thesis was conducted with OCs undertaking the CC at RMAS. Although the physical demands of this course have previously been documented, the nutritional intake of OCs in this setting has not been investigated during training in camp and during field exercises. Typically, in the military setting, due to the mismatch of EI to EE, a negative EB is often seen during periods of intense training such as field exercises. It is also reported that acute periods of negative EB are more beneficial to training adaptation if there is adequate recovery immediately after. However, it is unknown whether soldiers are in a positive or negative EB longitudinally during intense training.

In a military setting, differences in EE and EI have been quantified by calculating EB, but has not been quantified using the more contemporary approach of calculating EA. As such, few studies have explored this concept in military personnel and would likely be of benefit to understand the interaction between nutrition and adaptation to training in these populations.

The negative consequences that are linked to sub-optimal EB and EA have been found to be mitigated by nutritional strategies. One of which is the distribution of protein throughout the day every 3 - 4 hours, which can mitigate the loss of lean muscle mass in times of a negative EB by increasing MPS. Finally, there is limited research on the use of protein supplementation during military field exercises on EB, EA and physical performance.

Therefore, based on the review of literature, it is pivotal that the nutritional behaviour as well as the physical activity profiles of military personnel undergoing training is explored further to understand how nutrition can be tailored to those undergoing arduous training. Conducting this research, will inform the implementation of evidenced-based nutritional strategies to help enhance training, recovery and adaptation within camp field exercise training environments.

Therefore, the overall aims of this thesis (Figure 2.2) are to;

1. Quantify the physical activity profile of the 44-week Commissioning Course (CC) and determine the energy and macronutrient intake of OCs during training compared to current military and athletic guidelines (Study 1 - Chapter 4);
2. Quantify longitudinal and acute EB in OCs during the 44-week CC and to determine whether estimated body mass change agrees with actual body mass change over the acute periods (Study 2 - Chapter 5);
3. Investigate EA in OCs undertaking arduous training during the 44-week CC (Study 3 - Chapter 6);

4. Determine differences in the daily distribution of nutritional intake in relation to physical activity in three different military settings; on camp only, during field exercises and combined camp and field training (Study 4 - Chapter 7);
5. Investigate the effect of dietary protein supplementation on energy and macronutrient intake and muscle function and soreness during an arduous military field exercise (Study 5 - Chapter 8).

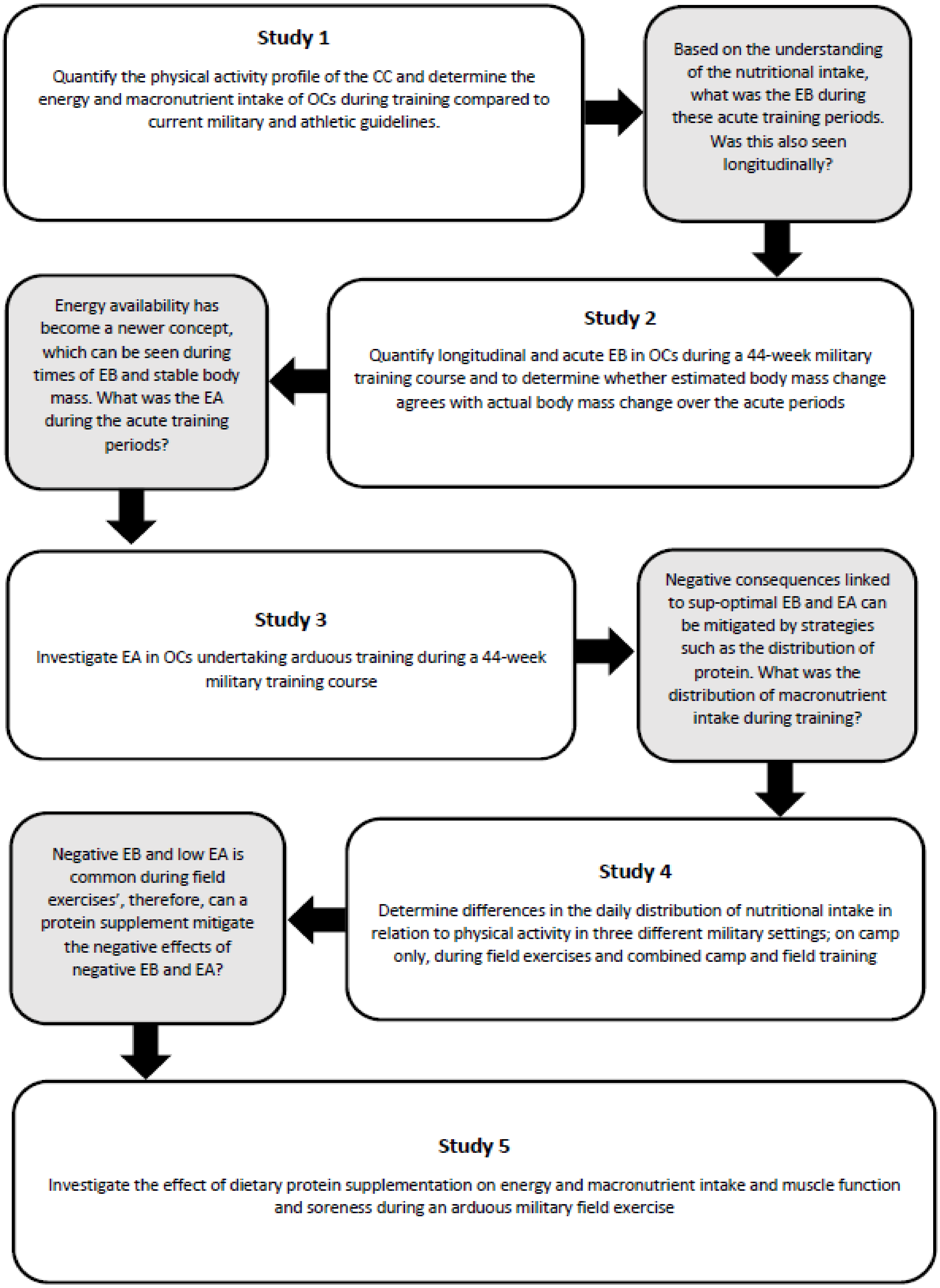

Figure 2.1: *Overview of aims (white boxes) and proposed questions (grey boxes) in Studies 1 to 5 within the thesis*

Chapter 3

General Methods

General Methods

This section provides a detailed description of the methods that have been used in the experimental chapters of this thesis. This includes a review the nature, history, use and applicability of the methods used and gives further detail on how they were employed in the present thesis.

3.1 Energy Expenditure and Physical Activity

Measuring the activity profile and energy demands of different populations is important to inform evidence-based research and development, particularly in the military (Horner et al., 2013). Accurately measuring Energy Expenditure (EE) in the military, ensures that physical training and military field exercises can best match the physical demands experienced by soldiers, and can inform military feeding strategy's in camp and in the field (Horner et al., 2013). As well as understanding the total daily energy requirements, an understanding of the frequency, intensity, type and timing of physical activity in physically demanding occupations is needed to help prevent injury while maintaining performance (Redmond et al., 2013). Previous tools that have been used to quantify EE include self-report questionnaires, logs, diaries, pedometers, accelerometers, heart rate monitors, and direct observation (Redmond et al., 2013). Direct observation is considered the most valid, reliable and objective method for assessing physical activity (Malina et al., 2004). However, it is also labour-intensive and introduces some subjectivity of activity intensity, and therefore other appropriate measuring tools may need to be used depending on research aims.

3.1.1 *Indirect calorimetry*

Energy expenditure was first measured in humans via direct calorimetry around 1900 (Westerterp, 2013), which specifically measured the rate at which an individual dissipates heat (Snellen, 2000). However, due to the method being expensive and difficult to operate, direct calorimetry has been replaced by indirect calorimetry (Westerterp, 2013), which relies on the measurement of the metabolic conversion of food energy with oxygen to create carbon dioxide. This can be calculated from the volume of oxygen consumed and carbon dioxide produced (Westerterp et al., 1984). Examples of systems that use this method are; respiratory chambers, ventilated hood systems (Westerterp, 2013) and Douglas bags or facemasks (Brychta et al., 2010). However, outside the laboratory, these methods are difficult to use due to their restriction on day-to-day activities, and therefore challenges arise for free-living measurements, such as limited monitoring duration, poor compliancy and lack of concurrency (Brychta et al., 2010). Further methods have therefore been introduced to measure free-living EE, which are discussed in the following sections.

3.1.2 *Doubly Labelled Water*

Doubly labelled water (DLW) was developed to be used in animals in 1955, and it wasn't until 1982, that it was first used in humans (Westerterp, 2013) as the first measurement for free-living EE (Buchowski, 2014). Since then, it has been validated against other methods such as; Energy Balance (EB) and weighed intake to determine if EE from DLW differed from EI when in a stable body mass (Schoeller & Van Santen, 1982), 3-day respiratory gas exchange measured Energy Intake (EI) in a respiratory chamber (Westerterp et al., 1984), and near-continuous gas exchange using a facemask that was worn continuously throughout the day apart from meal times (Schoeller, 1988; Schoeller et al., 1986; Schoeller & Webb, 1984). The DLW technique is suggested to be the gold-standard to measure total EE in free-living conditions (Park et al., 2014).

The DLW method uses heavy isotopes of oxygen (^{18}O) and hydrogen (^{2}H), which are elements that occupy the same position in the periodic table as oxygen and hydrogen, respectively. The isotopes are essentially chemically and functionally identical, but differ in mass due to a different number of neutrons within the atomic nucleus (Wilkinson, 2018). This difference in mass makes these isotopes analytically distinguishable from each other, therefore, if introduced into a system the metabolic fate of these isotopes can be traced (Wilkinson, 2018).

The fundamental basis of the DLW method is that oxygen turnover in the body is dominated by the flow of water as well as inspired oxygen and expired carbon dioxide (Speakman, 1998). The turnover of body hydrogen, however, is dominated only by the flow of water through the body, consequently meaning the difference between the turnovers of oxygen and hydrogen provides a measure of excess efflux of oxygen that is equivalent to the production of carbon dioxide (Speakman, 1998) (Figure 3.1).

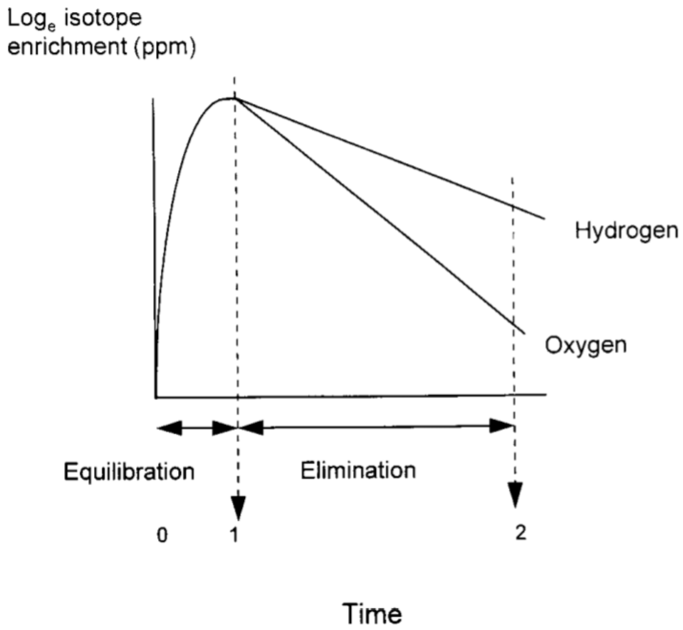

Figure 3.1: *The theoretical time course of enrichments of isotopes of oxygen and hydrogen in body water after administration of Doubly Labelled Water (DLW) at time zero (Speakman & Racey, 1988)*

Lifson and McClintock (1966) observed that the isotope, ^{18}O, in water is rapid and can complete isotopic equilibrium with the O_2 in CO_2. Therefore a water molecule labelled with ^{18}O will not only mix with body water and exit the same way as ^2H-labelled water, but will mix with CO_2 and exit the body as CO_2 (Park et al., 2014). Over a period of days, the difference of loss of ^{18}O and ^2H from the body reflects the rate at which CO_2 is produced, which in turn can be used to estimate EE based on the CO_2 production rate and respiratory quotient (Weir, 1949).

In its simplest form, the application of DLW involves the dosing of a participant with the isotope, sample collection, sample analysis and the calculation of EE (Westerterp, 2017). Once the participant is dosed, body water enrichment for ^{18}O and ^2H can be measured in blood, saliva or urine, and are subject to different degrees of isotope fractionation (thus are not combined in the same protocol) (Prentice, 1990). Water from blood, saliva, or urine samples are converted or equilibrated with H_2 and with CO_2, and the enrichment of the two gases are measured with isotope ratio mass spectrometry (Westerterp, 2017). Serum was considered the medium of choice for a long time, due to its rapid tracer equilibration (Schoeller & Van Santen, 1982). Saliva samples have also been shown to be an acceptable medium for measurement of Total Body Water (TBW) using $^2H_2{}^{18}O$, as isotopic equilibration occurs within 3 hours and has been shown to provide results similar or even identical to those obtained in serum (Lukaski & Johnson, 1985; Schoeller et al., 1982; Wong et al., 1988). Jankowski (2004) investigated the within-participant comparisons of the time to achieve a $_{2}$H enrichment plateau in saliva, urine, and serum samples obtained simultaneously. The results showed that the time to equilibrium varied among the three media, with the enrichment plateau achieved earliest in serum (1.5 hours), followed by saliva and urine (2.0 and 2.5 hours, respectively), and the decrease in enrichment began earlier in saliva (4.0 hours) than in urine or serum (5.0 and 6.0 hours, respectively). Although all three mediums have been shown to be acceptable in research, the usual choice of sample is usually urine or saliva due to being less intrusive and burdensome to participants. Urine is usually chosen for adult studies, however, a precaution must be taken to ensure that the urine has not been collecting in the bladder over a long period since it will then be impossible to ascribe an accurate time to the sample (Prentice 1990). Therefore, in a field environment, both urine and saliva seem reasonable choices of medium, with saliva being the more obvious choice when in a remote setting where lavatory facilities may not be often present.

In healthy adults, isotope elimination is determined by collection of samples at the beginning and end of an observation, however it can also be preferred to use the multipoint method (Cole & Coward, 1992), where samples are collected on a daily basis to improve the precision of the measurement and reduce the analytical variation. However, it must be noted that there might be day-to-day differences, albeit generally small differences, in isotope elimination rate due to daily variation in EE (Welle, 1990).

Schoeller and Van Santen (1982) were the first to validate DLW for a measurement of EE in free-living humans. The study was measured on four young adults, one women and three men, over a course of 14 days, and was compared with EE calculated though dietary weighing and the change in body stores calculated through TBW. Energy expenditure measured by DLW differed in comparison with EI and changes in body stores by 2.1 ± 5.6%. The same authors, also validated EE by the DLW method with simultaneously measured EE using a respiratory chamber, and found there to be a precision of 2 - 8% (Schoeller, 1988). Other validation studies were typically performed with simultaneous measurements of carbon dioxide production in a respiration chamber, and have shown an accuracy within 2% with a coefficient of variation of 2 - 12% (Schoeller, 1988; Speakman, 1998; Westerterp et al., 1984). These validation studies have included sedentary conditions, as well as measurements at high-activity levels, and have demonstrated the utility of the DLW method for the determination of carbon dioxide production in the range of activity levels in daily life (Westerterp, 2017).

Although DLW is known to be the gold-standard method, the technique provides a mean estimate of EE over a series of days, and lacks on providing minute-by-minute EE data, therefore cannot provide Physical Activity EE (PAEE) (Dannecker et al., 2013). Physical activity is described as "any bodily movement produced by the contraction of skeletal muscle that increases EE above a resting level" (Butte et al., 2012). Therefore, estimating PAEE, rather than absolute daily EE can be necessary to determine adequate dietary intake around activity.

The DLW method is used in Study 2 (Chapter 5) and Study 3 (Chapter 6).

3.1.3 *Activity Monitors*

Within field studies, wearable activity monitors have become an effective tool for measuring physical activity (Chowdhury et al., 2017). This is primarily due to their low cost, non-invasive and instantaneous feedback (Patel et al., 2015). Most commercially available activity monitors rely on movement sensors alone (accelerometers), however more recent models have integrated heart rate sensors to improve the estimation of EE (Brage et al., 2004; Strath et al., 2005). Additionally, algorithms have been validated to detect certain types of activity, and therefore can precisely estimate EE (Dannecker et al., 2013).

Tri-axial accelerometers are capable of sensing acceleration along the vertical, anterior-posterior and medio-lateral axes which is then categorised into sedentary, light, moderate and vigorous zones (Freedson et al., 1998; Hendelman et al., 2000). Validation studies have found that these high grade research devices can be used to objectively, unobtrusively and accurately identify physical activity, as well as provide detailed information about intensity and duration of physical activity (Redmond et al., 2013). Research-grade activity monitors have demonstrated varied success in the measurement of EE in free-living subjects when compared to the DLW method, with the explained variance from multivariate models ranging from 0.12 to 0.86 (Jeran et al., 2016). Previous research using activity monitors in the military setting, have cautioned that carrying load over varying terrain, which is often included in military training, may induce a higher EE as the same movement frequency's (*i.e*. walking and running) (Kinnunen et al., 2012). However recent research by Siddall, Powell, et al. (2019) on Officer Cadets (OCs) over 10 days has shown that using research grade accelerometers (GENEActiv (Original), Activ insights Ltd., Cambridge, UK) demonstrated a mean bias (± limits of agreement) of -15 ± 851 $kcal \cdot d^{-1}$ between DLW, with a linear correlations of 0.79 between the two methods. These findings suggest that the use of the GENEActiv research grade activity monitor has practical suitable and sufficient accuracy for use in military settings where personnel are experiencing high external and internal loads. Additionally, physical activity monitors have shown efficacy when distributed to large military cohorts for physical demands monitoring (Horner et al., 2013; Redmond et al., 2013). Therefore, evidence suggests that using a research-grade device could be valid for measuring gross EE and, given its lower demand for specialist expertise and comparative cost, maybe sufficiently accurate and practical to use in the military as a suitable alternative to DLW (Siddall, Powell, et al., 2019).

Tri-axial accelerometers are used in Study 1 (Chapter 4), 4 (Chapter 5) and 5 (Chapter 8).

3.2 Dietary Intake

The emergence of DLW to estimate free-living EE, enabled habitual EI to be estimated based on the concept of EB, that under conditions of weight stability, EE must equal EI (Magkos & Yannakoulia, 2003; Martin et al., 2009; Schoeller et al., 1990). However, in studies when participants may be in energy deficit, or where nutritional and macronutrient breakdown of the diet is to be explored, this method may not be suitable. Therefore, other methods have been used extensively to obtain real time and accurate estimates of food intake.

Obtaining real-time and accurate estimates of food intake whilst participants reside in their natural environment, is technically and methodologically challenging (Martin et al., 2009). Most studies that involve nutritional intake evaluation, involve either a retrospective method; 24-hour recall, food frequency questionnaires, diet history, and methods based on narrative accounts of food consumed in the past; or prospective methods such as food diaries, food weighing and direct observation (Gittelsohn et al., 1994). These methods have been fraught with long acknowledged limitations.

3.2.1 *Retrospective methods*

Food frequency questionnaires, which assess nutritional intake by asking the respondents about the frequency of usually consumed foods, are popular within large research projects, as they have minimal participant and researcher burden (Haftenberger et al., 2010). Within large studies or surveys, this method can give a broad representable overview of the nutritional intake and health within a specific population (Haftenberger et al., 2010; Heaney et al., 2010), and is usually employed over 6 months, denoting it beneficial for middle or long-term periods. However, for studies that are monitoring participants over periods of days to weeks, with the objective of quantifying specific nutritional day-to-day intake, this method is not feasible.

Alternatively, the dietary recall method is a subjective, retrospective method that requires the participant to precisely recall, describe and quantify the intake of foods and beverages consumed within a precise measurement period (Castell et al., 2015). However, like the food frequency questionnaire, it is also designed for large cohorts and captures 'usual' intake (Castell et al., 2015), and therefore does not capture day-to-day variation. Although the dietary recall method has certain benefits, such as being inexpensive and requires minimal participant burden, the practicality is not feasible in settings where participants have busy lifestyles, where their daily intake is likely to change often, or within a dining hall facility such as the military setting (Pouladzadeh et al., 2014).

The estimates of calorific intake derived from retrospective methods in comparison to unbiased biomarkers of EI such as DLW, have been shown to be significantly biased in the direction of underreporting for food frequency questionnaires, 24-hour dietary recalls and food records by approximately 12 - 20% (Hill & Davies, 2001; Shook et al., 2018; Subar et al., 2003; Trabulsi & Schoeller, 2001). Therefore, for smaller population studies, these retrospective methodologies are subject to random error due to poor recollection of diet, as well as systematic error due to underreporting the true value (Trabulsi & Schoeller, 2001).

3.2.2 *Prospective methods*

In comparison to retrospective methods, prospective methods, such as food diaries, food weighing and direct observation, have been shown to be advantageous in that they are not subject to recall inaccuracy of food eaten or inaccurate reporting (Gittelsohn et al., 1994). However, despite having greater accuracy, some prospective methods can be considered obstructive and intrusive, and may alter dietary behaviour (Gittelsohn et al., 1994).

Food dairies are by far the most common dietary assessment tool that is applied within sports nutrition research and practice (Capling et al., 2017). This method relies on participants recording food and drink as they are consumed, over a specified duration. Although this method is popular, self-reported intakes have shown to be underestimated by 37% in comparison to methods such as DLW (Goris et al., 2000), which appears to be constant across population groups, with younger men and women underreporting by 18 - 23% (Livingstone et al., 1990; Martin et al., 1996; Seale & Rumpler, 1997), elderly subjects by 12 - 31% (Goran & Poehlman, 1992), lean women by 18% (Lissner et al., 1989), and athletes by 13 - 35% (Edwards et al., 1993; Westerterp et al., 1986). Underestimations can be attributed to user measurement error, such as conscious or sub-conscious exclusion of foods (Capling et al., 2017; Howat et al., 1994; Magkos & Yannakoulia, 2003), under- or over-estimation of portion sizes, which tend to be underestimated when portions are large and overestimated when portions are small (Beasley et al., 2005; Bingham, 1987; Goris et al., 2000), and change in habitual intake due to increased subject burden or low self-awareness with regard to food intake (Magkos &

Yannakoulia, 2003). These participant errors have been apparent in adolescents (Livingstone et al., 1990), obese individuals (Goris et al., 2000; Prentice et al., 1989) and recreational / elite athletes (Hill & Davies, 2001; Westerterp et al., 1986).

In order to exclude any implausible food diaries, Goldberg and Black (1998) determined a cut off value to evaluate whether reported EI was a valid estimate of actual intake during a period of investigation. The cut off value would statistically show that if the mean reported EI to Basal Metabolic Rate (BMR) ratio (EI:BMR) is less than the lower 95% confidence limit, that is highly improbable that the reported intake could represent a genuinely low intake obtained by chance (Goldberg & Black, 1998). Although food diaries, combined with the Goldberg Cut off, can remove a large amount of bias, the population group needs to be considered. A suitable mean cut off can be used based on the individuals level of activity which may range from light to heavy (Goldberg & Black, 1998). However, within extreme cases, which has been demonstrated within military studies (Hoyt et al., 2006), EI can be extremely low, and therefore would be considered to be underreported even within this highest cut off range. Therefore, in occupational settings, especially during field exercise or operational deployment, this method would prove infeasible as it may be difficult to differentiate between individuals who misreport and those with extremely low (yet truthful) EI.

Within the military, when personnel are supplied with rations, validation studies on self-reported food intake demonstrated an agreement of > 95% with DLW (DeLany et al., 1989), which was suggested to be due to the participants having pre-supplied food, and therefore taking away the need for dietary recall. On the contrary, in settings where soldiers are able to eat *ad libitum*, self-reported intake has been shown to be under-reported by 23 - 33% (Burstein et al., 1996). Unfortunately, under these conditions, it seems that under-reporting may be inevitable when using food diaries, as the tight schedules, that are often seen in military training facilities may not always allow for organised meals, and therefore participants may be focused on matters other than the accurate compiling of a food diary (Hill & Davies, 2001).

Dietary assessment by direct observation and food weighing is generally accepted as the ‘gold-standard’ in civilian (Biro et al., 2002) and athletic (Magkos & Yannakoulia, 2003) populations. The method, which involves visually estimating or weighing the food item(s) served, as well as weighing any discards, has been shown to be most accurate (Gittelsohn et al., 1994). Within a population setting where participants eat within a canteen style environment, dietary weighing can be easily employed, however, it is well known that these methods are slow, labour intensive and produce participant and researcher burden (McClung et al., 2017).

Accurate assessment of dietary intake is, and will continue to be, a challenge for researchers within the field and non-laboratory setting (McClung et al., 2017). Therefore, it is important that the methods employed to measure EI are carefully considered in order to obtain a balance between measurement error and participant burden.

Food diaries and dietary weighing were used in Study's 1 - 5 (Chapters 4 - 8).

3.3 Body Composition

A variety of techniques have been used to measure body composition in civilian, athletic and military populations, such as underwater weighting, bio-impedance analysis, Duel Energy X-ray Absorptiometry (DXA), and TBW which all differ in various criteria, including sensitivity, cost, precision, invasiveness and technical complexity (Lee & Gallagher, 2008). These methods are regularly used to assess body composition, however are limited by the generalised assumption(s) that must be applied to the entire population (Van Der Ploeg et al., 2003). As *in vivo* techniques do not measure body composition directly, but rather predict it from measurements of body properties, all techniques suffer from two types of error: methodological error when collecting raw data, and error in the assumptions by which raw data are converted to final values. The relative magnitude of these errors varies between techniques (Wells & Fewtrell, 2006).

3.3.1 *Total Body Water*

Water is the major chemical component of the body and the essential medium of the bodies internal environment, whereby approximately 65% of the TBW is intracellular with 35% extracellular water in the proverbial 70 kg human (Chumlea et al., 1999). Total body water volume compromises approximately 50 - 60% of adult body mass with a range from 45 - 75% depending on sex, age and levels of muscle or Fat Mass (FM) (Chumlea et al., 1999). Fat mass is free of water, whereas the hydration of Fat Free Mass (FFM) is relatively constant in healthy adults, decreasing from 80% at birth to 73% at adulthood (Westerterp, 2017). However this exact value of a healthy adult can reportedly fluctuate ± 5% daily, due to ongoing physiological processes and the consumption of food and drink (Chumlea et al., 1999).

Measuring TBW in humans using the isotope dilution technique, is the most accurate method of measuring the bodies water pool (Wishart, 2011). The use of deuterium to estimate TBW was initially applied to measure body composition using a two compartment model (FM and FFM) (Pace & Rathbun, 1945). The basic principle underlying the technique, is that the volume of a body composition compartment can be defined by the dose of the tracer relative to its concentration in that compartment within a specific time period following dose administration (Wishart, 2011). The method used is the same as that described in Section 2.7.1.2 for DLW, whereby a single bolus dose of hydrogen (deuterium ^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H_2{}^{18}O$) is administered orally, which diffuses freely through all body compartments with no permeability barrier (Watson et al., 1980). Total body water is then determined through semi-log extrapolation to the time of dose administration (slope-intercept method), using the deuterium enrichments at certain time points (Van Marken Lichtenbelt et al., 2007). This, therefore, allows FFM to be calculated from TBW (Schoeller et al., 1980), and FM to be calculated as the difference between body mass and FFM (Wells & Fewtrell, 2006; Wishart, 2011). Validation studies have shown that TBW compared with underwater weighing, resulted in a correlation coefficient of 0.97 - 0.99 (Van Marken Lichtenbelt et al., 2007), and has shown to be an accurate and precise, free of bias method compared with the four compartment model (Ramirez et al., 2009). One issue associated with deuterated water is that the hydrogen dilution volume is greater than the body's water space because of the exchange with labelled hydrogen of protein and other body constituents (Schoeller et al., 1980). This results in an overestimation of TBW, therefore the exact error is debatable but is generally estimated to be between 1 - 5%. However, it must be noted, that many of these studies validating TBW are conducted in specific populations such as children or ethnic groups, therefore data in athletes or lean individuals are limited.

Body composition measured via TBW is used in Study 2 (Chapter 5) and Study 3 (Chapter 6).

3.3.2 *Duel-energy X-ray Absorptiometry*

Duel-Energy X-ray Absorptiometry is a non-invasive method that provides a direct measure of body composition based on a three compartment model: FM, FFM and bone density (Lee & Gallagher, 2008). This method has been considered the gold-standard for the measurement of body composition (Gupta et al., 2011), and has been shown to be an effective measure that is not dependent on age, sex, race and health status (Van Der Ploeg et al., 2003).

Body composition is calculated by ascertaining the amount of radiation attenuated by the bone or soft tissue, while low and high-energy radiograph beams are transmitted on the participant (Chertow, 1999; Pupim et al., 2004). This technique requires the participant to lie on the examination surface as a scanning arm performs a series of transverse movements emitting photons at the two energy levels. The soft tissue mass reduces photon flux much less than bone mineral density, therefore differential absorption of the photons is measured and processed by the DXA software to estimate bone mineral density, FM, and FFM (Pupim et al., 2004).

Validation studies have determined DXA to be the most accurate non-invasive measure of body composition and has a precision error of 0.5 - 3.0% and an accuracy error of 3.0 - 9.0% (Fogelholm & Van Marken Lichtenbelt, 1997; Pupim et al., 2004), where the range is dependent on population, BMI and age (Pupim et al., 2004). For example, comparisons have been made by measuring young or old groups (Clasey et al., 1999; Prior et al., 1997) and sedentary populations (Gallagher et al., 2000). Only few studies have investigated the use of DXA against a reference method in active lean individuals (Van Der Ploeg et al., 2003), where DXA progressively underestimated the body fat percentage of leaner individuals compared with the four compartment model (Van Der Ploeg et al., 2003). Other studies, have reported similar results in healthy adults, demonstrating that the DXA underestimates body fat percentage (0.4 - 4.2%) compared with other body composition methods such as underwater weighing, TBW, and bio-impedance analysis (Friedl et al., 1992; Fuller et al., 1992; Gallagher et al., 2000). For longitudinal studies, DXA been deemed a reliable measurement, such as examining the weight gain / loss in settings such as clinical or athletic populations, and has shown a reproducibility of 0.74 - 0.95 (Lohman et al., 2009). Although DXA provides a precise measurement of body composition, there are still considerable concerns about its validity, especially hydration level (Pietrobelli et al., 1998) which has been calculated to have an error of < 0.1% when measuring body fat due to hydration changes of 1 - 5%. One study examined the body fat of healthy male soldiers who lost > 10% of body mass over an 8-week Army Ranger Course, whilst remaining highly active during a large energy deficit. The study found that DXA significantly overestimated tissue mass towards the end of the course, which is speculated to be due to a misinterpretation of hydration assumptions (Friedl et al., 1994), and therefore should be used with caution in a military population.

Body composition measured via DXA is used in Study 5 (Chapter 8).

3.4 Muscle Function

In the military, the ability to generate force and power is of primary importance (Comfort & Pearson, 2014), due to large number of critical occupational tasks involving muscular strength (Hauschild et al., 2016), however during times of arduous exercise and / or body mass loss, physical performance has been shown to be reduced (Nindl et al., 2007). Therefore, it is necessary to accurately evaluate an individual's performance after periods of exercise using valid and reliable assessment methods (Nikolaidis et al., 2019). To quantifiably assess strength, a variety of means have been used, such as isometric tests or isokinetic tests (McLean & Conner, 1994). In the military, dynamics tests have been used such as a deadlift, which reflects the ability to lift and move equipment or personnel from the ground. However, isometric tests may be preferred to dynamic tests for their reduced injury risk, relatively simple administration and high test-retest reliability (Beckham et al., 2013). They have also been found to be advantageous for defining true levels of changes in strength (Buckner et al., 2017), and can provide indirect evidence of muscle damage after exercise (Warren et al., 1999). However, it must be noted, that peak force and torque measured after exercise can be influenced by central and metabolic muscle fatigue and thus may not be useful for identifying the extent of the muscle damage (Nosaka et al., 2006).

3.4.1 *Isometric Mid-Thigh Pull*

Many occupations in the military require soldiers to possess a certain physical capacity to safely and effectively perform occupation tasks (Chasse et al., 2019), which often include explosive power through the lower and upper extremities (Stockbrugger & Haennel, 2001). Predicting performance is therefore essential for determining physical capabilities of trainee soldiers (Chasse et al., 2019). The Isometric Mid-Thigh Pull (IMTP) has been shown to be a relatively simple technique that may be an alternative to dynamic tests such as One-Repetition Maximum (1RM) lifts (Brady et al., 2018), due to its relatively quick administration and reduced injury risk (Brady et al., 2018).

The test is designed to replicate the body's position at the beginning of the second pull of the clean or snatch (Haff et al., 1997), which has been shown to be the strongest and most powerful position during weightlifting movements, and generates the highest force and velocity of any part of the lift (Garhammer, 1993). The IMTP is performed standing on a force platform, that quantifies the vertical ground reaction forces (Brady et al., 2018), inside a custom-designed power rack that allows fixation of the bar at any height. The participant is positioned so that they maintain an upright trunk position (Haff et al., 1997), with the knee angle between 130^{o} and 140^{o} (Haff et al., 2008) and a hip angle of 124^{o} and 175^{o} (Beckham et al., 2013). Video analysis of lifting performance in elite weightlifters, has previously determined the positioning of the knees and hips to be the most appropriate angles to produce the greatest amount of force when conducting the isometric test (Brady et al., 2018; Haff et al., 2005). Once the participants has assumed the body position described, the bar height is adjusted to the height of the mid-thigh (Thomas et al., 2015) and the participant is then able to utilises a clean grip (Gourgoulis et al., 2002). Once stabilised, the participant is asked to provide minimal tension to the bar to ensure that there is no slack in the body prior to the initiation of the pull (Beckham et al., 2013). Instructions are then given to pull against the bar with maximal effort as quickly as possible (Beckham et al., 2013; Thomas et al., 2015). The test is often repeated multiple times and the trial with the highest value is used, in order to ensure that the data represent actual physiological values as much as possible and to exclude learning and motivational effects (De Witt et al., 2018).

Peak force determined from the IMTP has shown to be strongly correlated to performance on dynamic tests such as counter movement jump peak power (r = 0.72 - 0.79) (Mcguigan et al., 2010; Stone et al., 2004), Wingate peak power (r = 0.74) (Stone et al., 2004), 1RM back squat (r = 0.97), 1RM bench press (r = 0.99) (Mcguigan et al., 2010), 1RM deadlift (r = 0.88) (De Witt et al., 2018) and weightlifting competition performance in snatch and clean and jerk (r = 0.82) (Beckham et al., 2013) in both recreationally active individuals and athletes.

3.4.2 *Isokinetic Dynamometry*

Within the military, training is characterised by a high level of load carriage (Pihlainen et al., 2017), which requires a high level of metabolic and mechanical energy, which taken together with other physiological stressors, can induce neuromuscular fatigue (Grenier et al., 2012). Neuromuscular fatigue is defined as an exercise-related decrease in the maximal strength or power of a muscle, whether or not the task can be sustained (Grenier et al., 2012), and has shown to be impaired after load carriage in men (O'Leary et al., 2017).

The gold-standard for measuring muscle function in clinical practice and research settings is isokinetic dynamometry, which provide a constant velocity with accommodating resistance throughout a joint's range of motion (Drouin et al., 2004). Within the military, the ability to produce torque quickly, can provide an indication of functional strength (Miller et al., 2006). Furthermore, using continuous concentric-eccentric test cycles, can reflect physiological activities during which different muscle actions are employed, rather than isolated concentric or eccentric actions (Chi Tin Li 1996). Therefore using isokinetic dynamometers within the occupational setting can allow for the evaluation of muscle function, enabling subjects to perform maximum contraction throughout the full range of motion without the risk of injury (Brown, 2000).

Isokinetic dynamometry measurements are usually performed unilaterally on the right side, and participants are seated with a strap applied across the hips and shoulders to stabilise the participant and to avoid additional movement (Van Driessche et al., 2018). Following warm up repetitions, maximal concentric isokinetic contractions are performed. The peak torque measured by isokinetic dynamometers, gives the highest muscular force output at any moment during a repetition, and is ideally suited for examining changes in muscle strength at different velocities before and after an experimental intervention (Montgomery et al., 1989). Therefore, it is demonstrated to be a reasonable option for assessing strength measurements in the military following arduous exercises (Dempsey et al., 1998).

Although the technique is expensive and lacks portability (Toonstra & Mattacola, 2013), its test-retest reliability has shown to be high for measurements of peak torque with coefficients reported to be 0.95 - 0.97 on extension, and 0.82 - 0.99 on flexion (Feiring et al., 1990). Other studies have shown similar results and demonstrated Biodex dynamometry to be a highly reliable (knee flexion: ICC = 0.93 - 0.98, knee extension: ICC = 0.96 - 0.97) and valid measure of strength (Drouin et al., 2004; Keskula et al., 1995; Sole et al., 2007).

The use of IMTP and Biodex dynamometry are used in Study 5 (Chapter 8).

Chapter 4

Study 1

Dietary Intake and Physical Activity Profile of Officer Cadets during Military Training Compared to Military and Athletic Guidelines

4.1 Abstract

Background: Evidence-based nutritional guidelines have been developed for use in military settings, however it is unknown whether Army Officer Cadets (OCs) meet these guidelines during training. The aim of this chapter is to quantify the physical activity profile of OCs throughout the Commissioning Course (CC) and compare the dietary intake of OCs during training to current guidelines. **Method:** Twenty-six participants (14 men and 12 women) were observed during training in Camp (CAMP), on a Field Exercise (FEX) and during a combination of both camp and field training (MIX). The physical activity profile of the course was measured continuously using a tri-axial accelerometer from which Energy Expenditure (EE) was estimated. Dietary intake of all core meals was measured through researcher led dietary weighing and collection of all food wrappers. Dietary intake was analysed using nutritional analysis software (Nutritics, Nutritics LTD, Ireland) to calculate energy and macronutrient intake. A two-way Analysis of Variance (ANOVA) was used to compare means of weekly EE between sex and term (Junior, Intermediate and Senior Term), as well as total Energy Intake (EI) between sex and condition (CAMP, FEX and MIX). Paired sample t-tests compared EI against Military Dietary Reference Values (MDRVs) and relative carbohydrate and protein intake against athletic guidelines. **Results:** Officer Cadets had a greater average EE (mean ± SD; men: 4708 ± 334 and women: 3822 ± 292 $kcal \cdot d^{-1}$) during Junior Term, compared to Intermediate (men: 4266 ± 228 and women: 3406 ± 269 $kcal \cdot d^{-1}$; $p < 0.001$) and Senior (men: 4002 ± 152 and women: 3345 ± 270 $kcal \cdot d^{-1}$; $p < 0.001$) Term. Energy intake was lower than the MDRVs (4600 and 3500 $kcal \cdot d^{-1}$ for men and women, respectively) for CAMP (men: 3763 ± 890 and women: 3077 ± 449 $kcal \cdot d^{-1}$; $p < 0.05$), FEX (men: 3279 ± 661 and women: 2043 ± 420 $kcal \cdot d^{-1}$; $p < 0.001$) and MIX (men: 3009 ± 443 and women: 2410 ± 296 $kcal \cdot d^{-1}$; $p < 0.05$). Mean relative carbohydrate intake was below the minimum requirement of athletic guidelines (< 6 $g \cdot kg \cdot d^{-1}$) for both men and women during FEX (men: 4.1 ± 1.0 and women: 3.7 ± 1.3 $g \cdot kg \cdot d^{-1}$; $p < 0.05$) and MIX (men: 4.0 ± 0.7 and women: 3.9 ± 1.2 $g \cdot kg \cdot d^{-1}$; $p < 0.05$), however did not differ from the guidelines for CAMP (men: 5.4 ± 1.7 and women: 5.4 ± 1.0 $g \cdot kg \cdot d^{-1}$; $p > 0.05$). Mean relative protein intake was greater than the minimum athletic guidelines (> 1.2 $g \cdot kg \cdot d^{-1}$) for men and women during CAMP (men: 1.7 ± 0.3 and women: 1.6 ± 0.3 $g \cdot kg \cdot d^{-1}$; $p < 0.001$), however did not differ from the requirements for MIX (men: 1.3 ± 0.2 and women: 1.2 ± 0.2 $g \cdot kg \cdot d^{-1}$; $p > 0.05$). During FEX, protein intake did not differ from the guidelines for men (1.1 ± 0.2 $g \cdot kg \cdot d^{-1}$; $p > 0.05$), however were below the guidelines for women (0.9 ± 0.2 $g \cdot kg \cdot d^{-1}$; $p > 0.05$). **Conclusion:** During military training, energy and macronutrient intake may be suboptimal when considering athletic and military based guidelines.

4.2 Introduction

High physical activity levels have been reported during British Army Officer Cadet (OC) training (Bilzon et al., 2006). Energy and macronutrient intake is essential for individuals to meet, and adapt to, the demands of training (Beals et al., 2015), enhance and / or maintain physical and cognitive performance (McClung & Gaffney-Stomberg, 2016; Moran et al., 2012) and reduce risk of fatigue, injury and illness (Rodriguez et al., 2009). During hard physical training, energy and macronutrient needs, especially carbohydrate and protein, must be met to maintain body mass, replenish glycogen stores, and provide adequate protein to build and repair muscle tissue (Rodriguez et al., 2009).

The nutritional guidelines for the British military (Military Dietary Reference Values; MDRVs) provide guidance on appropriate energy and macronutrient intake for military personnel in different scenarios (Hill et al., 2011). There is no direct evidence in the military population evaluating the impact of different macronutrient sources on performance, meaning the UK macronutrient guidelines for military personnel are largely based upon evidence from athletic populations undertaking sports training and competition. The most recent guidelines recommend OCs consume 4600 (men) and 3500 (women) kcal·d^{-1} during the Commissioning Course (CC) (SACN, 2016). The macronutrient recommendations for OCs are based on providing adequate nutritional needs to meet the demands of training, and are dependent on total Energy Intake (EI), and are therefore set as a percentage of the total energy intake stated in the MDRVs (50 - 65% of total EI from carbohydrate, 10 - 15% from protein and 25 - 35% from fat) (Gillen et al., 2017; SACN, 2016).

Recent reviews suggest that macronutrient recommendations as a percentage of EI are not the most desirable way of expressing intakes and, instead, protein and carbohydrate should be determined per kilogram of body mass (Burke et al., 2001; Phillips, 2012). This is to account for individual variation as well as activity type, duration and intensity (Thomas et al., 2016). Athletic guidelines for protein intake, suggest that requirements for individuals with high training loads are likely to exceed the general population guidelines of 0.8 g·kg·d^{-1} (Gillen et al., 2017; IOM, 2000), with suggested doses of 1.2 - 2.0 g·kg·d^{-1} (Pasiakos, Montain, et al., 2013; Phillips et al., 2007; Phillips & Van Loon, 2011; Rodriguez et al., 2009). Protein intake at the higher end of the recommendation may optimise the desired adaptive response to long-term training such as potentiating muscle gain through increased net protein balance, improving key performance indicators such as strength and power, and aid muscle recovery by increasing Muscle Protein Synthesis (MPS) to repair and replace damaged proteins (Phillips & Van Loon, 2011; Rodriguez, 2013; Rodriguez et al., 2009). Athletic guidelines for carbohydrate intake range from 3 - 12 g·kg·d^{-1} for athletes, with the higher intakes (> 6 g·kg·d^{-1}) recommended for long duration, high intensity exercise (Potgieter, 2013). Higher carbohydrate intake is necessary to maintain / enhance performance when undertaking endurance exercise, high intensity interval training and long duration field exercises (DeBolt et al., 1988). Conversely, low carbohydrate intake is associated with reduced soldier performance on military tasks (Jacobs & Sherman, 1999; Montain et al., 1997). It is also important after exercise, to enhance recovery and optimise glycogen stores for subsequent training sessions (Potgieter, 2013).

General guidelines for the public suggest that fat intake should be 25 - 35% of total EI in ensure optimal health, maintain Energy Balance (EB) and to ensure optimal intake of essential fatty acids and fat soluble vitamins (Potgieter, 2013). During times when Energy Expenditure (EE) is high but available time to eat is restricted, typically the case during military field exercises, energy dense foods, such as fat, may protect against large energy deficits (Tharion et al., 2004). However, in sports performance settings an excessive consumption of fat has been discouraged due to the concern that it may displace carbohydrate rich foods, which are necessary for moderate-to-high intensity exercise (Price et al., 1994), and thereby prevent adequate carbohydrate intake (Burke et al., 2004).

The British Army CC is a 44-week long course, broken down into three 14-week terms, Junior, Intermediate and Senior Term, where each is separated by one week of adventure training and two-to-three weeks of leave. During the CC, men and women OCs train together in integrated platoons. The course is comprised of classroom and practical lessons and physical fitness through physical training lessons, which include activities such as plyometric exercise, interval training, resistance training, simulated occupational tasks (including loaded marches), and self-selected sports on a weekly basis. Due to the high energy demands of the CC, it is important that OCs' dietary intake meets dietary guidelines (Tharion et al., 2004), to help optimise physical and cognitive performance (McClung, 2017), reduce injury risk (McClung, 2017) and promote recovery and adaptation during training (Beals et al., 2015). Therefore, the aims of this study were twofold: 1) to quantify the physical activity profile of the whole CC and 2) determine the energy and macronutrient intake of OCs during training compared to current military and athletic guidelines.

4.3 Method

4.3.1 *Participants*

Twenty-six participants (14 men and 12 women) volunteered for the study and participant characteristics are shown in Table 4.2. Fourteen participants (seven men and seven women) volunteered for all three conditions, an additional five participants (three men and two women) volunteered for two conditions and a further seven participants (four men and three women) volunteered for one condition. Participants were treated as independent samples between each condition. Ethics approval was granted by the Ministry of Defence Research Ethics Committee (protocol number 780/MoDREC/16). All participants were provided with a written and verbal brief on the requirements of the study and offered the opportunity to ask questions before providing written informed consent.

4.3.2 *Study Design*

The general approach to this study was to measure dietary intake during three contextually different periods of the CC (Weeks 9, 22 and 34). The first period was during nine days training in camp (CAMP) during Junior Term, where the OCs undertook classroom-based work, rifle and combat drills and instructor led physical training. During this time, participants ate in the dining hall, where they were supplied hot food, or on the ranges, where they were supplied a field meal or a packed lunch. Personal food items were also permitted to be eaten. The second period was during five days on a defensive Field Exercise (FEX) consisting of constant low-to-moderate activity, digging, and limited sleep. Participants were supplied 24-hour ration packs and were also permitted to bring any other non-perishable items, and eating was *ad libitum*. The third period was nine days of combined camp and public-order field based training (MIX) where participants were based in camp for three days where they undertook normal training such as that in CAMP and ate in the dining hall, and six days on a riot-based exercise, where they acted as both civilians and riot patrol. Participants ate in a field-based kitchen, similar to that of the dining hall, as well as bringing their own items of food.

4.3.3 *Physical Activity Profiling*

Energy expenditure over the training course was estimated using a wrist-worn tri-axial accelerometer (GENEActiv, Activinsights, UK), which has previously been demonstrated to be a reliable and valid measure of EE (Esliger et al., 2011; Plasqui et al., 2005). Accelerometers have been successfully used to monitor physically demanding occupations without causing undue burden upon participants (Blacker et al., 2009; Richmond et al., 2014). The GENEActiv devices were set at a sampling frequency of 50 Hz and calibrated to each participant’s sex, age, height and body mass (measured at the beginning of each sampling block) and were worn at all times throughout the year, except for outside term time. Accelerometers were collected and re-issued on a fortnightly basis. Raw acceleration data were analysed to estimate Metabolic Equivalents (METs), using a macro in a Microsoft Excel spreadsheet supplied by Activinsights, and summed to calculate MET minutes (MET·mins^{-1}). Any minute with a zero value was replaced with 0.9 METs to reflect a low baseline of estimated resting metabolism; data (per day) were deemed invalid and excluded from analysis if the device was worn < 65% of the day. Daily EE was calculated using MET·mins^{-1} and participant body mass (kg) using Equation 4.1a (Bushman, 2012). To increase the accuracy of EE and adjust to the military population, measurements from the GENEActiv, a linear regression was applied to the results, using Equation 4.1b (Blacker et al., 2019).

$$a) \qquad EE = MET.mins\ x\ 3.5\ \times\ BM\ /\ 200$$

$$b) \qquad Adjusted\ EE = 563.116 + (0.886\ \times EE_{GENEActiv})$$

Equation 4.1: *Calculation of hourly estimated Energy Expenditure (EE) (a) and adjusted EE (b)*

4.3.4 *Dietary Intake*

Dietary intake of all core meals were primarily measured through researcher led dietary weighing in the military dining hall and food diaries as a secondary source when the researcher was not with the participants. In CAMP and MIX, dietary weighing was conducted by researchers placed at three locations in the dining hall and field kitchen; the hot plate, salad bar and dessert stand. When participants entered the dining hall they were provided with a tray marked with their participant number. At the hot plate, a 'protein portion' was served via dining hall staff, which was subsequently weighed. The participants were then permitted to help themselves to all other items, which were each individually weighed. Upon finishing, all discards, including any wrappers from sauces, food and drinks, were weighed. Participants were provided with food diaries on a daily basis to record all food eaten per day, and a small zip-lock bag to contain wrappers from any food consumed. Participants were also asked to record the time of day the food was eaten, the brand, the location (e.g. dining hall, shooting range etc.) and the portion size. During FEX, OCs were required to store all rations wrappers and any additional food item wrappers in zip-lock bags, with the day and time eaten, written on the wrapper. All wrappers were collected each day by the researchers and discards were weighed. The dietary intake of each participant was analysed using nutritional analysis software (Nutritics, Nutritics LTD, Ireland) to calculate energy and macronutrient intake.

4.3.5 *Data Analysis*

Three participants withdrew from FEX due to injury. Participants were treated as independent groups, whereby 20 participants were included in CAMP, 17 were included in FEX, and 20 were included in MIX. Data (per day) were excluded if participant's food intake was not weighed and / or if they did not fully complete the food diary for any single day. Participants were questioned about any food diaries that lacked the required information, and dismissed if the information could not be reliably gathered. Across the three data collection periods a total of 390 of a possible 492 food diaries were completed. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS; IBM SPSS version 23 for Windows, IBM Corporation, Chicago, IL). Results are expressed as mean ± one standard deviation. Statistical significance was set *a priori* at $p < 0.05$. Data were assessed for normality to ensure that kurtosis and skewness were within the normal bound, and where appropriate, non-parametric analyses were conducted and homogeneity of variances was confirmed using Levene's test. Data were analysed as absolute values for total daily energy and macronutrient intake, as well as relative to body mass for carbohydrate and protein. A two-way Analysis of Variance (ANOVA) with reported effect size (partial eta squared [η^2_p]) was used to compare means of weekly EE between sex (men and women) and term (Junior, Intermediate and Senior Term), as well as total EI intake between sex and condition (CAMP, FEX and MIX). Dietary data were analysed using paired sample t-tests with reported effect sizes (Cohen's d) to compare the mean energy intake against the MDRVs and to compare relative carbohydrate and protein intake against the minimum requirement of athletic guidelines. Interpretation of Cohen's d is as follows: ≤ 0.2 trivial effect , 0.21 - 0.50 small effect, 0.51 - 0.80 moderate effect and ≥ 0.8 a large effect (Cohen, 1988). Significant interaction effects were analysed with Bonferroni.

4.4 Results

4.4.1 *Participant Characteristics*

There was no difference in participant age ($F_{(2, 54)} = 2.419$, $p = 0.099$, $\eta^2_p = 0.082$), body mass ($F_{(2, 54)} = 0.324$, $p = 0.725$, $\eta^2_p = 0.012$), or height ($F_{(2, 54)} = 0.145$, $p = 0.866$, $\eta^2_p = 0.005$) between conditions.

4.4.2 *Termly Physical Activity Profile*

For average weekly EE (Figure 4.1) there was a main effect of term ($F_{(2,77)} = 34.82$, $p < 0.001$, $\eta^2_p = 0.475$), irrespective of sex, where weekly EE was higher during Junior Term compared to Intermediate (mean difference [95% CIs]: 429 [246 - 611], $p < 0.001$, d = 0.818) and Senior (628 [444 - 812], $p < 0.001$; d = 1.295) Term. Weekly EE was also greater during Intermediate Term compared to Senior Term (199 [15 - 383], $p = 0.035$, d = 0.443). Men had a greater weekly EE compared to women ($F_{(1,77)} = 153.34$, $p < 0.001$, $\eta^2_p = 0.666$; Table 4.1), irrespective of term. Maximal daily EE over the year was 7163 (men) and 5582 (women) $kcal \cdot d^{-1}$, measured during week 8.

Table 4.1: *Average weekly Energy Expenditure (EE) of men and women during Junior, Intermediate and Senior Term*

		Energy Expenditure (kcal·d^{-1})	
		Men	**Women**
Average Term			
	Junior	4708 ± 334	3822 ± 292
	Intermediate	4266 ± 228	3407 ± 269
	Senior	3930 ± 307	3345 ± 270
Average Year		4301 ± 431	3529 ± 346

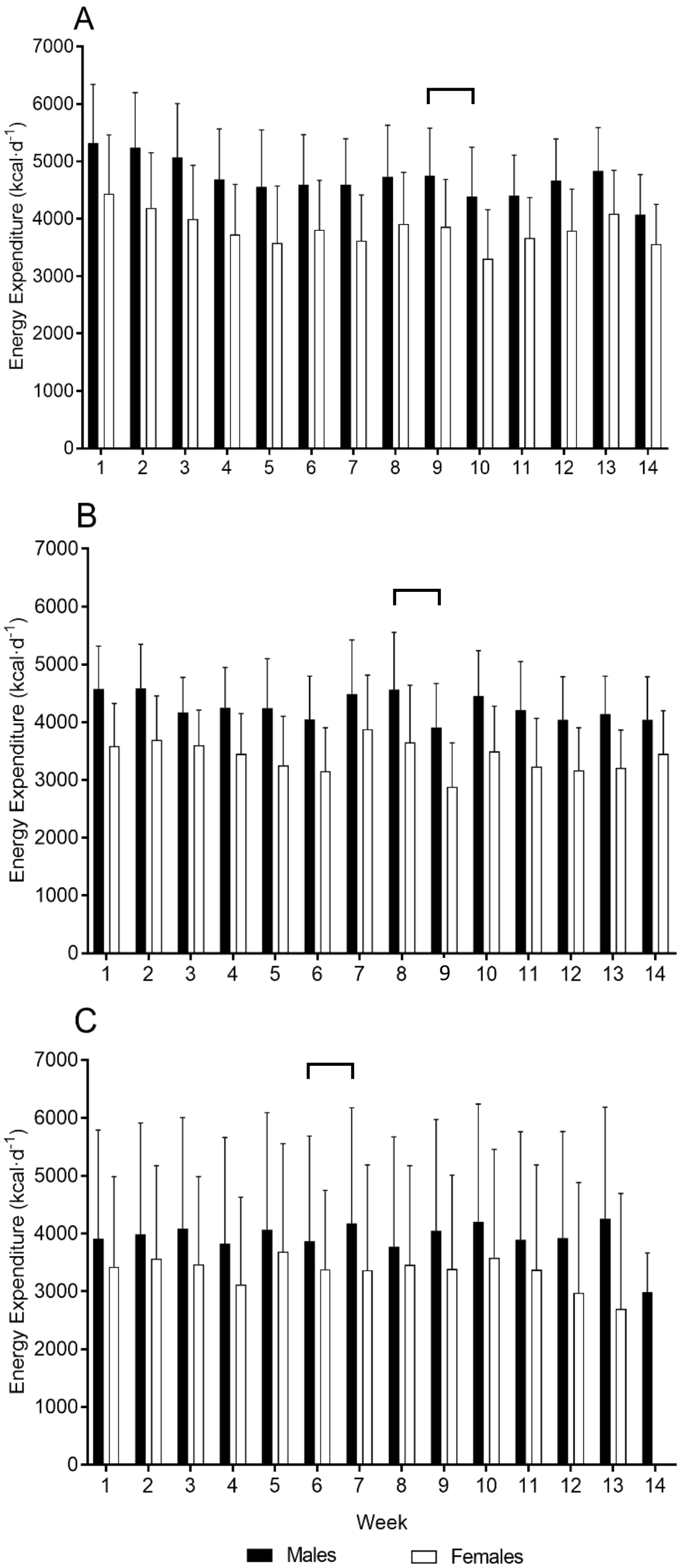

Figure 4.1: *Estimated weekly Energy Expenditure (EE) of men (■) and women (□) during Junior (A), Intermediate (B) and Senior (C) Term. The brackets denote the data collections for camp training (CAMP; week 9 in Junior Term), field exercise (FEX; week 8 in Intermediate Term) and a mix of both camp and field training (MIX; week 6 in Senior Term)*

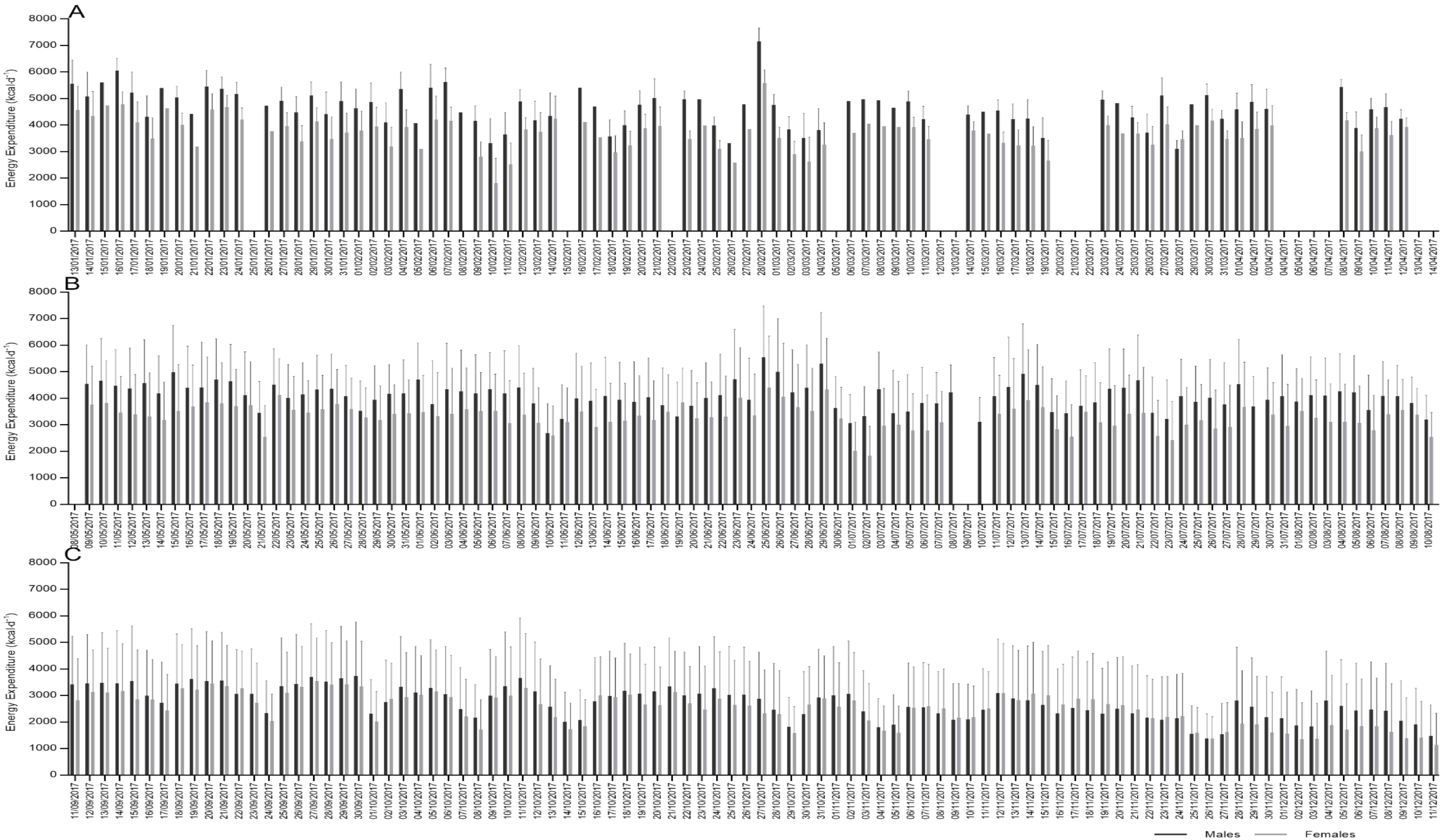

Figure 4.2: *Estimated daily Energy Expenditure (EE) to map the physical activity profile of men (■) and women (□) during Junior (A), Intermediate (B) and Senior (C)*

4.4.3 *Energy Intake*

For EI, there was a main effect of condition ($F_{(2,51)} = 10.98$, $p < 0.001$, $\eta^2_p = 0.301$; Table 4.3), irrespective of sex, where EI was greater during CAMP than FEX (mean difference [95% CIs]: 759 [309 - 1209], $p < 0.001$, $d = 0.947$) and MIX (710 [280 - 1141], $p < 0.001$, $d = 1.107$), but there was no difference between FEX and MIX ($p = 1.00$, $d = -0.073$). Men had a greater EI compared to women ($F_{(1,51)} = 31.39$, $p < 0.001$, $\eta^2_p = 0.381$), irrespective of condition. There was no interaction effect of sex and condition ($F_{(2,51)} = 1.677$, $p = 0.197$, $\eta^2_p = 0.062$). Energy intake was lower than the MDRVs for all conditions, where the mean differences were shown in Table 4.2. The EI of men was 80%, 67% and 58% of the MDRVs in CAMP, FEX and MIX, respectively. The EI of women was 87%, 47% and 63% of the MDRVs in CAMP, FEX and MIX, respectively.

Table 4.2: *Mean difference and 95% Confidence Intervals (95% CIs) between energy intake and military recommended dietary values during camp training (CAMP), on a field exercise (FEX) and combined camp and field training (MIX)*

	CAMP	FEX	MIX
Men			
Mean Difference	837 kcal·d^{-1}	1321 kcal·d^{-1}	1591 kcal·d^{-1}
95% CI	201 - 1473	814 - 1829	1274 - 1908
p	0.016	< 0.001	0.003
Women			
Mean Difference	424 kcal·d^{-1}	1457 kcal·d^{-1}	1090 kcal·d^{-1}
95% CI	102 - 745	1106 - 1809	878 - 1301
p	0.015	< 0.001	< 0.001

Table 4.3: *Participant characteristics, Energy Intake (EI), Protein (PRO) intake, Carbohydrate (CHO) intake, and fat intake, during camp training (CAMP), on a field exercise (FEX) and combined camp and field training (MIX)*

	CAMP			FEX			MIX		
Variables	**All**	**Men**	**Women**	**All**	**Men**	**Women**	**All**	**Men**	**Women**
n	20	10	10	17	9	8	20	10	10
Age (years)	23 ± 2	23 ± 2	22 ± 2	24 ± 2	24 ± 2	24 ± 3	24 ± 3	24 ± 2	24 ± 3
Height (m)	1.74 ± 0.08	1.90 ± 0.08	1.68 ± 0.04	1.75 ± 0.08	1.80 ± 0.07	1.69 ± 0.04	1.74 ± 0.08	1.79 ± 0.06	1.68 ± 0.05
Mass (kg)	77.0 ± 9.3	84.2 ± 6.4	69.8 ± 5.4	76.6 ± 9.9	83.7 ± 7.2	68.6 ± 5.0	78.9 ± 9.0	86.1 ± 6.6	71.7 ± 3.7
EI ($kcal \cdot d^{-1}$)	3420 ± 771	3763 ± 890	3077 ± 449	2697 ± 837*	3279 ± 661	2043 ± 420	2709 ± 478*	3009 ± 443	2410 ± 296
CHO Intake ($g \cdot d^{-1}$)	412 ± 101	449 ± 123	375 ± 59	353 ± 111	428 ± 93	268 ± 51	311 ± 63*	343 ± 63	279 ± 47
PRO Intake ($g \cdot d^{-1}$)	128 ± 29	143 ± 31	113 ± 17	79 ± 24*	94 ± 17	62 ± 19	101 ± 18*†	113 ± 15	89 ± 13
Fat Intake ($g \cdot d^{-1}$)	134 ± 33	148 ± 36	120 ± 24	105 ± 38*	129 ± 33	78 ± 24	112 ± 20*	124 ± 19	100 ± 11
CHO Intake ($g \cdot kg \cdot d^{-1}$)	5.5 ± 1.5	5.4 ± 1.7	5.4 ± 1.0	4.5 ± 2.0*	4.1 ± 1.0	3.7 ± 1.3	4.0 ± 1.2*	4.0 ± 0.7	3.9 ± 1.2
PRO Intake ($g \cdot kg \cdot d^{-1}$)	1.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.3	1.0 ± 0.6*	1.1 ± 0.2	0.9 ± 0.2	1.3 ± 0.3*†	1.3 ± 0.2	1.2 ± 0.2
Fat Intake ($g \cdot kg \cdot d^{-1}$)	1.7 ± 0.4	1.7 ± 0.3	1.7 ± 0.4	1.0 ± 0.3*	1.1 ± 0.2	0.9 ± 0.3	1.3 ± 0.2*	1.3 ± 0.2	1.2 ± 0.2

* significant difference of all participants from CAMP, † significant difference of all participants from FEX, $p < 0.05$.

4.4.4 *Carbohydrate Intake*

For absolute carbohydrate intake, there was a main effect of condition ($F_{(2, 51)} = 8.58$, $p < 0.001$, $\eta^2_p = 0.252$; Table 4.3), irrespective of sex, where participants consumed more carbohydrate on CAMP compared to MIX (mean difference [95% CIs]: 101 [41 - 160], $p < 0.001$, $d = 1.201$), however, there was no difference in carbohydrate intake between CAMP and FEX ($p = 0.050$, $d = 0.603$) or FEX and MIX ($p = 0.458$, $d = 0.423$). Men had a greater intake of carbohydrate compared to women ($F_{(1,51)} = 22.99$, $p < 0.001$, $\eta^2_p = 0.311$), irrespective of condition. There was no interaction effect between sex and condition ($F_{(2,51)} = 2.10$, $p = 0.076$, $\eta^2_p = 0.076$).

For relative carbohydrate intake, there was a main effect of condition ($F_{(2,51)} = 12.01$, $p < 0.001$, $\eta^2_p = 0.320$), irrespective of sex, where intake was greater in CAMP compared to FEX (mean difference [95% CIs]: 0.9 [0.1 - 1.6], $p = 0.019$, $d = 0.805$) and MIX (1.5 [0.7 - 2.2], $p < 0.001$, $d = 1.544$), however there was no difference between FEX and MIX ($p = 0.244$, $d = 0.662$). There was no main effect of sex ($F_{(1, 51)} = 2.25$, $p = 0.140$, $\eta^2_p = 0.042$) or an interaction effect between sex and condition ($F_{(2,51)} = 2.12$, $p = 0.131$, $\eta^2_p = 0.077$).

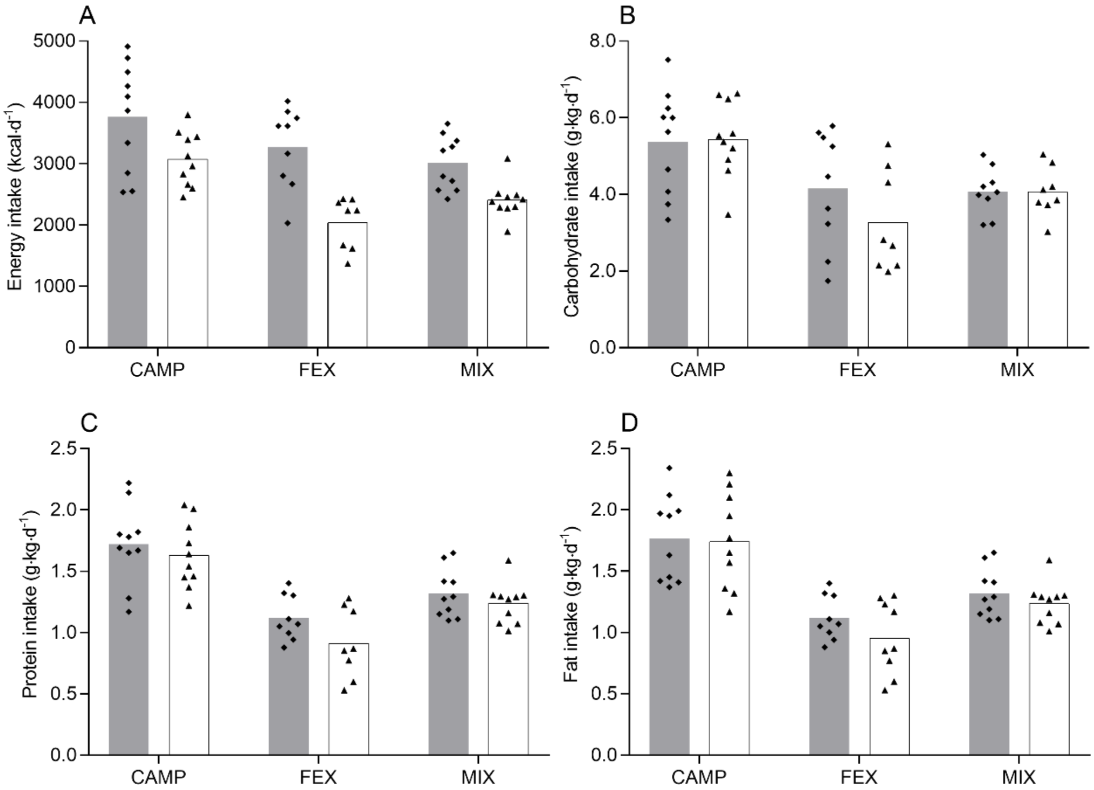

Figure 4.3: *Average energy (A), relative protein (B), relative carbohydrate (C) and relative fat (D) intake during camp training (CAMP), field exercise (FEX) and a combination of camp and field training (MIX) between men (■) and women (□) with individual data points overlaid*

During CAMP, mean relative carbohydrate intake did not differ from the minimum requirement of athletic guidelines (6 g·kg·d^{-1}) for men or women (Table 4.4). However, intake was below the minimum requirement of athletic guidelines for FEX and MIX.

Table 4.4: *Mean difference and 95% Confidence Intervals (95% CIs) between carbohydrate intake and athletic guidelines during camp training (CAMP), on a field exercise (FEX) and combined camp and field training (MIX)*

	CAMP	FEX	MIX
Men			
Mean Difference	0.6 g·kg·d^{-1}	0.9 g·kg·d^{-1}	2.0 g·kg·d^{-1}
95% CI	-0.3 - 1.6	0.2 - 1.6	1.5 - 2.5
p	0.180	0.014	< 0.001
Women			
Mean Difference	0.6 g·kg·d^{-1}	2.0 g·kg·d^{-1}	2.1 g·kg·d^{-1}
95% CI	-0.2 - 1.3	1.3 - 2.8	1.6 - 2.6
p	0.107	< 0.001	< 0.001

4.4.5 *Protein Intake*

For absolute protein intake, there was a main effect of condition ($F_{(2,51)} = 29.88$, $p < 0.001$, $\eta^2_p = 0.540$; Table 4.2), irrespective of sex, where intake in CAMP was greater than FEX (mean difference [95% CIs]: 50 [34 - 66], p < 0.001, d = 1.863) and MIX (27 [12 - 42], p < 0.001, d = 1.110), and intake on MIX was greater than FEX (23 [7 - 39], p = 0.003, d = 1.090). Men had a greater protein intake compared to women ($F_{(1,51)} = 30.42$, $p < 0.001$, $\eta^2_p = 0.374$), irrespective of condition. No interaction effect was seen ($F_{(2,51)} = 0.207$, $p = 0.814$, $\eta^2_p = 0.008$).

For relative protein intake, there was a main effect of condition ($F_{(2,51)} = 34.16$, $p < 0.001$, $\eta^2_p = 0.573$; Table 4.2), irrespective of sex, where CAMP was greater than FEX (mean difference [95% CIs]: 0.7 [0.5 - 0.9], p < 0.001, d = 2.381) and MIX (0.4 [0.2 - 0.6], p < 0.001, d = 1.615), and MIX was greater than FEX (0.3 [0.5 - 0.1], p = 0.006, d = 1.205). There was no main effect of sex ($F_{(1,51)} = 34.16$, $p = 0.059$, $\eta^2_p = 0.068$) nor an interaction effect ($F_{(2,51)} = 0.355$, $p = 0.703$, $\eta^2_p = 0.014$).

For men, mean relative protein intake was greater than the minimum athletic guidelines (1.2 g·kg·d^{-1}) during CAMP (Table 4.5) however did not differ from the requirements for FEX nor MIX. For women, mean relative protein intake was greater than the minimum athletic guidelines (1.2 g·kg·d^{-1}) during CAMP, and was below the guidelines for FEX, however did not differ from the guidelines for MIX.

Table 4.5: *Mean difference and 95% Confidence Intervals (95% CIs) between protein intake and athletic guidelines during camp training (CAMP), on a field exercise (FEX) and combined camp and field training (MIX)*

	CAMP	FEX	MIX
Men			
Mean Difference	0.5 g·kg·d^{-1}	0.1 g·kg·d^{-1}	0.1 g·kg·d^{-1}
95% CI	0.3 - 0.8	0.0 - 0.2	0.0 - 0.3
p	0.001	0.064	0.087
Women			
Mean Difference	0.4 g·kg·d^{-1}	0.3 g·kg·d^{-1}	0.0 g·kg·d^{-1}
95% CI	0.2 - 0.6	0.1 - 0.2	-0.2 - 0.1
p	< 0.001	0.011	0.492

4.4.6 *Fat Intake*

For absolute fat intake, there was a main effect of condition ($F_{(2,51)} = 7.08$, $p = 0.002$, $\eta^2_p = 0.217$; Table 4.3), irrespective of sex, where intake during CAMP was greater than FEX (mean difference [95% CIs]: 30 [10 - 51], p = 0.002, d = 0.859) and MIX (22 [2.3 - 41], p = 0.028, d = 0.804), however there was no difference between FEX and MIX (p = 0.948, d = -0.288). Men had a greater intake than women ($F_{(1,51)} = 25.78$, $p < 0.001$, $\eta^2_p = 0.336$), irrespective of condition, however there was no interaction effect between sex and condition ($F_{(2,51)} = 1.39$, $p = 0.258$, $\eta^2_p = 0.052$). For relative fat intake, there was a main effect between condition ($F_{(2,51)} = 33.85$, $p < 0.001$, $\eta^2_p = 0.570$), irrespective of sex, where CAMP was greater than FEX (0.7 [0.5 - 1.0], p < 0.001, d = 2.326), and MIX (0.5 [0.3 - 0.7], p < 0.001, d = 1.650), and MIX was greater than FEX (0.3 [0.0 - 0.5], p = 0.018, d = 1.209). There was no main effect of sex ($F_{(1, 51)} = 2.02$, $p = 0.162$, $\eta^2_p = 0.038$) nor an interaction effect ($F_{(2,51)} = 0.50$, $p = 0.608$, $\eta^2_p = 0.019$).

4.5 Discussion

The present study is the first to quantify the dietary intake for both men and women during the 44-week CC in relation to physical activity levels and compare these to military and athletic guidelines.

The weekly average EE measured in the present study (men: 4002 - 4708 and women: 3345 - 3822 kcal·d^{-1}) was similar to previous training courses in both British and international military populations as described in Section 2.1.2 in Chapter 2. For example, British Army Parachute Regiment recruits expended up to 4553 ± 571 kcal·d^{-1} during a 10-day period of training (Wilkinson et al., 2008), and OCs in the Royal Military Academy Sandhurst (RMAS) have reported EE as high as 4898 ± 430 in men and 3822 ± 478 kcal·d^{-1} in women (Bilzon et al., 2006). Between each term, average weekly EE progressively lowered, which reflects the structure of the CC, where Junior Term has a greater time spent doing physical training that is more rigid and structured, compared to Senior Term where more time is spent in academic classes and physical training is self-administered.

The estimated average EE in the current study were higher in Junior Term than the current military guidelines for both men and women, but lower in Intermediate and Senior Term. Averaged over the year, EE were in line with the MDRVs, and provides some evidence that they are useful as a guide to the overall energy requirements during OC training (SACN, 2016). It is evident from the present study that there were periods of training where EI did not reach the energy demands of activity. This, and its effect on energy balance, was not a research aim of the current chapter but will be explored later in this thesis (Study 2).

The second aim of the study was to explore the dietary intake of OCs to compare against current military and athletic guidelines. The main findings were that EI was lower than current military dietary guidelines (men: 4600 and women: 3500 kcal·d^{-1}) for all conditions, carbohydrate was lower than the guidelines (6 - 12 g·kg·d^{-1}) during FEX and MIX, and protein was lower than athletic guidelines for FEX (1.2 - 2.0 g·kg·d^{-1}). Previous research in the British Army demonstrated similar EI (men: 2846 ± 573 and women: 2207 ± 585 kcal·d^{-1}), carbohydrate (men: 4.8 ± 1.3 and women: 3.8 ± 1.4 g·kg·d^{-1}), and protein (men: 1.5 ± 0.3 and women: 1.3 ± 0.3 g·kg·d^{-1}) intake in recruits undergoing Phase-1 training (O'Leary et al., 2018). The reported under consumption observed in the present study is typical of that observed in previous research in the military population (Fallowfield et al., 2010; McAdam et al., 2018).

A common theme between training courses demonstrating high EEs, is that OCs are often physically active for a large part of the 24 days (Hoyt et al., 2001), sleep deprived (Shippee et al., 1994) and carrying external loads (Tharion et al., 2004). Despite the compressed timetable during the CC, three core meals times are scheduled each day, however, unless the OCs consume adequate energy in these three periods, there may be little time to supplement extra energy in-between meal times. It is also likely that, during field exercises, despite adequate provision of energy and macronutrients, trainees are often unable to match the required EI due to limited time to eat. In US military training courses, soldiers have been found to consume 56% (Margolis et al., 2016) and 50% (Margolis, Murphy, et al., 2014) of the supplied rations during exercise. Suboptimal intake may influence training outcomes, compromise the ability to recover from high volume training and decrease the desired adaptive response (Beals et al., 2015; Davey et al., 2011).

Military guidelines suggest that carbohydrate intake should be between 50 - 65% of total EI (SACN, 2016) which, when expressed as a relative intake based on an average sized man eating the required amount of energy (4500 kcal), equates to approximately 7 g·kg^{-1} of body mass. These Military recommendations therefore agree with athletic dietary guidelines which suggest that athletes who train between 1 - 3 hours a day, should consume 6 - 10 $g{\cdot}kg{\cdot}d^{-1}$ (Masson & Lamarche, 2016; Rodriguez et al., 2009). Repeated bouts of intense exercise on the same day or over several days without sufficient recovery, causes acute fatigue, or the inability to maintain exercise workloads (Meeusen et al., 2013). High intensity exercise is reliant on carbohydrate as an energy source to enhance exercise tolerance time and combat fatigue (Jeukendrup, 2014). Additionally, suboptimal intakes of carbohydrate during long-term training may impact the quality of training and recovery. Research has reported that lower carbohydrate intake impacts performance on military tasks (Jacobs & Sherman, 1999; Montain et al., 1997); soldiers who were restricted to ingesting 250 $g{\cdot}d^{-1}$ of carbohydrate for 4 days had a significant deterioration in shooting performance after a loaded 4-hour treadmill march compared to a group who ingested 400 - 550 $g{\cdot}d^{-1}$ (Tharion & Moore, 1993). The average relative carbohydrate intake of the OCs over the three conditions (CAMP: 5.5; FEX: 4.5; MIX: 4.0 $g{\cdot}kg{\cdot}d^{-1}$) was below athletic guidelines, and even more so during FEX. Despite these recommendations, during long physically active days, where military personnel are undertaking more sustained low-to-moderate intensity exercise combined with intermittent periods of high intensity exercise, the focus, where possible, may be primarily to focus on adequate carbohydrate around physical activity rather than ensuring absolute amounts. This has been shown in previous research, which demonstrated that 60 $g{\cdot}h^{-1}$ of carbohydrate, reduced perceived exertion and increased incidence of task completion during 3 hours of fixed-intensity loaded walking (Byrne et al., 2005). Therefore, if consuming an adequate amount of carbohydrate is not possible, especially during times of field exercise, it could be suggested that soldiers focus on timing carbohydrate intake strategically around exercise bouts rather than aiming for a specific total intake.

Military dietary guidelines recommend that protein intake is 10 - 15% of the total EI (SACN, 2016) and unlike carbohydrate, are fixed at the same percentage of EI, even when EE is increased. However, during periods of reduced caloric intake, existing evidence suggests potential benefits from higher protein intake (Ferrando, 2013). There is growing consensus that percentages of total EI from macronutrients are not always the most desirable way of expressing intakes and therefore recommendations for protein intake are expressed per kilogram of body mass (Burke et al., 2001; Phillips, 2012). More recently, evidence has been presented that endurance and resistance trained athletes should consume protein relative to body mass at an intake of 1.2 - 2.0 $g.kg{\cdot}d^{-1}$ (Pasiakos, Montain, et al., 2013; Phillips et al., 2007; Phillips & Van Loon, 2011; Rodriguez et al., 2009). Although the current UK government Recommended Dietary Allowance (RDA) is 0.8 $g{\cdot}kg{\cdot}d^{-1}$ of protein, there is broad agreement that this amount is insufficient to promote optimal health, and that individuals that are involved in strenuous endurance and resistance training, similar to that of OCs, may need a greater intake (1.5 - 1.8 g·kg·d^{-1}) of dietary protein than the current RDA (Mamerow et al., 2014; Tarnopolsky, 2004). A recent consensus statement recommended that soldiers should consume 1.5 - 2.0 g·kg·d^{-1} of protein to mitigate declines in whole-body protein balance, based on evidence demonstrating that consuming protein at a level twice the RDA promotes protein balance during short-term moderate energy deficit (Margolis, Murphy, et al., 2014; Pasiakos, Austin, et al., 2013). As a ratio of body mass, relative mean protein intake (CAMP: 1.7; FEX: 1.0; MIX: 1.3 g·kg·d^{-1}) in the present study was lower than the suggested intake (Tarnopolsky, 2004) for FEX and MIX but aligned to recommendations during CAMP.

Previous research on Norwegian soldiers found that during a 3-day ski march, despite being in a large energy deficit, soldiers consumed an optimal amount of protein (~1.7 g·kg·d^{-1}) according to athletic guidelines. However, interestingly, post-absorptive whole-body protein balance became progressively more negative throughout the exercise, suggesting an even greater intake of dietary protein may be needed the longer the operational task duration (Margolis, Murphy, et al., 2014). It has been reported that protein intake at levels twice the MDRVs spares whole-body protein balance during short-term energy deficits and therefore mitigates declines in Fat Free Mass (FFM) (Pasiakos, Cao, et al., 2013). This is likely due to the need for dietary protein intake to support the repair and remodelling of the skeletal muscle and connective tissue after exercise (Beelen et al., 2010). Work by Mettler et al. (2010) found resistance trained men in a 60% calorie deficit, consuming a higher intake of protein (2.3 g·kg·d^{-1}), lost less FFM compared to men consuming a lower intake of protein (1.0 g·kg·d^{-1}), despite equal total body mass loss between groups. However, despite these differences in FFM preservation, there were no differences in strength or muscular performance between groups, with higher protein intakes unable to preserve performance. A separate consequence of an energy deficit is a reduction of glucose production (Klein et al., 1993) due to hepatic glucose down-regulation. However, a study investigating the effects of increased protein intake during a 7-day period of a -1000 kcal·d^{-1} energy deficit, found that the group consuming 1.8 g·kg·d^{-1} of protein were able to maintain glucose production from glycogen breakdown (Smith et al., 2011), suggesting that the manipulation of dietary protein during an energy deficit may offset the associated fall in glucose production. Within the circumstances of an energy deficit, whether by EI or EE, evidence points to the potential benefits of an increased protein intake (Ferrando, 2013). However, despite this evidence in controlled laboratory based studies on short periods of training, there is limited research in military environments. Therefore, it is not unreasonable to suggest that in an operational setting, where exercise periods are longer in duration and energy deficits are greater, that even the highest protein recommendations may not be sufficient.

During FEX, participants consumed ration packs, which have an energy provision of 4000 kcal, consisting of 651 g carbohydrate, 130 g protein, and 92 g fat (Shaw & Fallowfield, 2013). Personnel are required to carry their own supplies and often discard any unwanted items. Therefore although an adequate amount of food is supplied to the OCs, it is unlikely that the whole ration pack is consumed (Tassone & Baker, 2017). The present study supported previous investigations that personnel undertaking field exercises are likely to experience periods of energy deficit. Lower nutritional intake during field exercises in the military setting are not uncommon and can provide opportunity for military trainees to understand potential physical and psychological stressors of operational deployment and prepare them for aspects of the stress of combat (Tharion et al., 2005). Pasiakos and Margolis (2017) state that in the context of the military task, some degree of energy deficit is expected and may be well tolerated as long as protein and carbohydrate intakes are consistent with recommendations.

Across all conditions, the recommended intake of fat (25 - 35% of EI) was reached by all participants (CAMP: 35; FEX: 34; MIX: 37%). Although carbohydrate and protein has been shown to be important for maintaining muscle mass (Calbet et al., 2017), recovery and improving performance (Jeukendrup, 2008, 2010; Moore et al., 2014; Phillips & Van Loon, 2011), lipids provide an energy potential twice that of carbohydrate and protein. When adapted to a low-carbohydrate high-fat diet, studies have demonstrated an increased fat oxidation rate, resulting in a reduced reliance on carbohydrate oxidation and ultimately sparing muscle glycogen (Phinney et al., 1983; Volek et al., 2016). Therefore, during field or operational exercises when soldiers may not have the time to meet energy demands, a higher fat intake than the recommended guidelines may spare such high energy deficits.

There were several limitations in the present study including the methods for measuring dietary intake, as well as the study only demonstrating a small proportion of the 44-week CC. Firstly, the present study used a combination of methods to measure EI, with dietary weighing used for the majority of main meals and food diaries to pick up any additional unobserved feeding. Direct observation has been shown to be more advantageous over other methods (Gittelsohn et al., 1994), as it involves researchers directly observing and weighing the food which the participant is eating, as well as weighing any discards. Despite it being the most stringent method researchers can employ to understand EI, it is labour-intensive and still contains limitations that likely influence accuracy such as using generic nutritional values for food items in the nutritional software. The likely accuracy of the EI measures during FEX are supported by validation studies on food intake in military personnel who are supplied with ration packs which have demonstrated an agreement of > 95% with Doubly Labelled Water (DLW) when in an energy balanced state (DeLany et al., 1989). Outside of dietary weighing, the retrospective recall through the food diary method is understood to have some inaccuracy due to conscious or sub-conscious exclusion of foods (Capling et al., 2017; Howat et al., 1994; Magkos & Yannakoulia, 2003) and / or under- or over-estimation of portion sizes (Beasley et al., 2005; Bingham, 1987; Goris et al., 2000), which may have influenced EI values. However, the researchers used a 'second pass' method, in which participants were asked for more detailed descriptions of the food consumed if it was thought that their food diaries were lacking in sufficient information. Therefore, the use of a food diary as well as dietary weighing for *ad libitum* intake seems a practicable balance of methods to more accurately depict how OCs eat when in training. Secondly, the three periods measured within the present study, though varied, are probably too short in duration to make interpretations of the 44-week course as a whole. As it would have been beyond the resources of the project to complete this data capture on many more occasions, it was thought that three short periods within three different military settings might be sufficiently representative of the broader training syllabus and would also not place too much burden on participants. The authors did also explore the option to estimate total daily EI based on the set menus provided by the dining hall, however it was quickly identified that the estimated intake was extremely different from what was actually consumed by the OCs.

4.6 Conclusion

In conclusion, the present study has demonstrated that the current military dietary guidelines are not consistently met in periods of arduous training, where EI were lower than the current MDRVs for all three contextually different training conditions examined. In comparison to athletic guidelines, which may arguably more closely match the nutritional requirements of military personnel than population guidelines, protein and carbohydrate intake were below the recommended intake during field exercise conditions. As such, this macronutrient intake may be inadequate to maintain / enhance performance and adaptation when undertaking endurance exercise and / or to optimise MPS to enhance recovery and adaptations to training, especially during prolonged periods of arduous military exercise. The addition of knowledge of dietary intake in different military settings can contribute to the design of nutritional strategies for the maintenance of health and performance of servicemen and women. Further research is warranted to explore EB and EA in field- and camp-based conditions, as well as exploring the strategic distribution of macronutrients around physical activity, in order to determine optimal nutritional strategies for military training.

The results from Study 1 demonstrate that EE remain consistently high throughout the CC, however these are not always matched by EI which may be reflected in the ability to maintain body mass during training. Therefore, Study 2 will aim to explore EB, and whether EI meets EE during longitudinal and acute periods, as well exploring changes in body mass and body composition of OCs in these periods.

4.7 Military Relevance and Recommendations

The outcome of this study will inform military personnel of the importance of meeting nutritional guidelines during camp and field training. During field exercise, military personnel should ensure energy intake meets or exceeds the guidelines (men: 4600 and women: 3500 $kcal \cdot d^{-1}$) and that protein intake should meet the requirement of 1.2 $g \cdot kg \cdot d^{-1}$. It would also be recommended that during periods of arduous exercise when time is limited to eat, that military personnel eat foods with higher content to meet their energy requirements. Ensuring that nutrition meets the recommended guidelines may allow military personnel to optimally adapt and recover during training.

Chapter 5

Study 2

Short-Term Negative Energy Balance Does Not Affect Longitudinal Energy Balance in British Army Officer Cadet Training

5.1 Abstract

Background: During military training, sustained periods of low-to-moderate physical activity, result in high daily Energy Expenditure (EE), that is difficult to match with Energy Intake (EI). An imbalance will result in positive or negative Energy Balance (EB), and a respective increase or decrease in an individual's body mass. However, it is unknown whether multiple acute periods of negative EB are representative of longitudinal EB in the military setting. The aims of this study were to (1) Quantify longitudinal EB over the Commissioning Course (CC) and acute EB during different military training settings of five to nine days' duration, and (2) compare changes in body mass estimated from EI and EE to directly measured changes in body mass during acute periods of training lasting five to nine days. **Method:** Thirteen Officer Cadets (OCs; six men and seven women; mean ± SD: age 24 ± 3 years, height 1.74 ± 0.07 m) volunteered to take part in the 44-week long study. Longitudinal EB was calculated via changes in body mass and body composition from the beginning to end of each term (Junior, Intermediate and Senior Term), as well as from the beginning to the end of the CC. Acute EB was calculated through EI, measured through dietary weighing, and EE, measured through Doubly Labelled Water (DLW), during Camp training (CAMP; nine days), Field Exercise (FEX; five days) and a combination of both camp and field training (MIX; nine days). Estimated body mass change, calculated from acute EB was compared to actual body mass change to determine the agreement. A repeated measures Analysis of Variance (ANOVA) compared longitudinal body composition as well as acute EB between each condition. Paired t-test determined body mass changes during each acute data collection period. **Results:** Longitudinally, body mass increased from start of Junior Term (75.1 ± 9.3 kg) to end of Senior Term (77.6 ± 9.3 kg; $p < 0.05$), demonstrating an overall positive EB during the entirety of the CC (49 ± 75 kcal·d^{-1}). Direct measurements of EE and EI indicated OCs were in multiple periods of acute negative EB (CAMP: -692 ± 506; FEX: -2190 ± 436; MIX: -1280 ± 309 kcal·d^{-1}), where FEX demonstrated the greatest negative EB ($p < 0.001$). Estimated body mass change calculated from acute EB (CAMP: -0.9 ± 0.6; FEX: -1.6 ± 0.3; MIX: -1.7 ± 0.4 kg), demonstrated no correlation to actual body mass change on CAMP (0.5 ± 1.5 kg; $p < 0.05$; $r = 0.179$), FEX (-0.7 ± 1.4 kg; $p < 0.05$; $r = 0.473$), and MIX (-0.2 ± 0.9 kg; $p < 0.001$; $r = 0.929$). **Conclusion:** Methods of measuring EB, whether by body mass changes, or through dietary assessment are highly variable. Therefore, it is recommended that observing and assessing trends in EB and body mass should be undertaken both acutely and longitudinally to offer greater insight into the physiological consequences of military training. Although the overall demands of the CC appear to be tolerable to most OCs, further investigation is warranted to elucidate the physiological consequences of repeated short-term bouts of negative EB, that may not be indicated by body mass, due to intense sustained physical activity.

5.2 Introduction

Officer Cadet training at the Royal Military Academy Sandhurst (RMAS) is a 44-week course, involving both on-camp training as well as UK and overseas exercises, which is known to be necessarily arduous. Due to the continuous nature of military work and training consisting of intermittent periods of moderate-to-high intensity exercise, combined with periods of prolonged low intensity exercise (Henning et al., 2011; Skiller et al., 2005; Tharion et al., 2005; Wilkinson et al., 2008), it is common for Officer Cadets (OCs) to experience periods of high Energy Expenditure (EE) (Bilzon et al., 2006). Energy expenditure has been reported to be as high as 6851 kcal·d^{-1} during an operational exercise and 5480 kcal·d^{-1} when training in camp (Margolis, Murphy, et al., 2014). Camp versus field environments have previously been shown to elicit different EE due to the abundance of ambulatory activities, carrying external load and longer workdays (Tharion et al., 2005). However, during these periods, EE is often difficult to match with (Energy Intake (EI), as food supply and time to eat or prepare meals are often limited (Margolis et al., 2013; Nindl et al., 2007). A mismatch between EI and EE can result in positive or negative EB, and a respective increase or decrease in an individual's body mass (Silva et al., 2017), which is accounted for by a gain or loss in Fat Mass (FM) and / or Fat Free Mass (FFM) (Forbes, 1987; Hall, 2007).

Consequently, large energy deficits have been shown to impair bone health (Klesges et al., 1996), increase injury rates (Dixon & Fricker, 1993; Schlabach, 1994) and compromise physical performance in opposition with desired military training outcomes (Montain & Young, 2003). Subsequently, negative Energy Balance (EB) has been a challenge for the military for many years, and remains a contemporary concern. The decrease in body mass associated with shorter periods (3 - 7 days) of negative EB in the military setting is primarily water loss (Margolis et al., 2016; Margolis, Murphy, et al., 2014), whereas decreases in body mass over longer training periods (weeks-to-months) of near continuous activity may be more detrimental to both physical and cognitive performance (Friedl, 1995; Pasiakos & Margolis, 2017). Small day-to-day variations in EB are unlikely to result in significant body mass changes that would be detrimental to performance, since it is typical for EI to be naturally matched by free-living EE in the intervening and subsequent days (Edholm & Fletcher, 1955). Large energy deficits would need to be sustained over longer periods of time (such as a minimum exercise intervention of two weeks) to result in detectable changes in body composition (Westerterp, 2018) which, in turn, could result in measurable decrements to physical performance (Pasiakos & Margolis, 2017). Therefore, the time domain in which a person is in negative EB, and the ability to accurately monitor longitudinal EB, need consideration in the design and practice of these studies (Hall et al., 2012). Likewise, it is unknown whether multiple acute periods of negative EB is representative of longitudinal EB in the military setting. Unfortunately, despite advances in technology, measuring EB is still technically and methodologically challenging, especially within a field-based or military environment. This is, in part, due to the cost of EE measurements such as Doubly Labelled Water (DLW), inaccuracies associated with quantifying EI, particularly recall of food diaries and underreporting, and the duration over which EB is measured (Westerterp, 2018). As such, the aim of this study was threefold; 1) To quantify longitudinal EB from changes in body mass and composition over each term (three 14-week periods) as well as over the year (44-week period), 2) to quantify acute EB over three different military settings from dietary weighing and DLW, and 3) to determine whether estimated body mass change, calculated from acute EB, agrees with actual body mass change over the acute periods.

5.3 Method

5.3.1 *Participants*

Fifteen participants (7 men and 8 women; age 24 ± 3 years, height 1.74 ± 0.07 m), who completed all three data collection periods, volunteered to take part in the year-long study. Participants were provided with a verbal and written brief on the requirements of the study, in the absence of any uniformed staff, and offered the opportunity to ask questions before providing informed written consent. Ethical approval was granted by the Ministry of Defence Research Ethics Committee (protocol number 780/MoDREC/16).

5.3.2 *Study Design*

The general approach to this study was to measure acute and longitudinal EB during military training. The Commissioning Course (CC) is structured into three terms of 14 weeks, with 2 - 4 weeks of holiday leave between terms. The first term (Junior Term; weeks 1 - 14) focuses on technical soldiering skills, physical fitness development and decision-making. The second term (Intermediate Term; weeks 15 - 29) focuses on development of leadership skills, and has a major academic component. The third term (Senior Term; weeks 30 - 44) focuses on implementing the main skills the OCs have developed in practice. All three terms incorporate academic classroom-based lessons, gym-based personal training, skill-based drills and weapon handling, and demanding operational exercises.

The acute data collection periods were measured over Camp training (CAMP), Field Exercise (FEX) and a combination of both camp and field training (MIX), as described in Section 4.3.2 in Chapter 4. Acute EB was measured via EI from dietary weighing and food diaries, and EE from DLW (Figure 5.1). Longitudinal EB was measured over each term via changes in body mass, FM and FFM (estimated using DLW) as well as over the entirety of the course estimated from changes in body mass. Participants undertook their normal training throughout the data collection period.

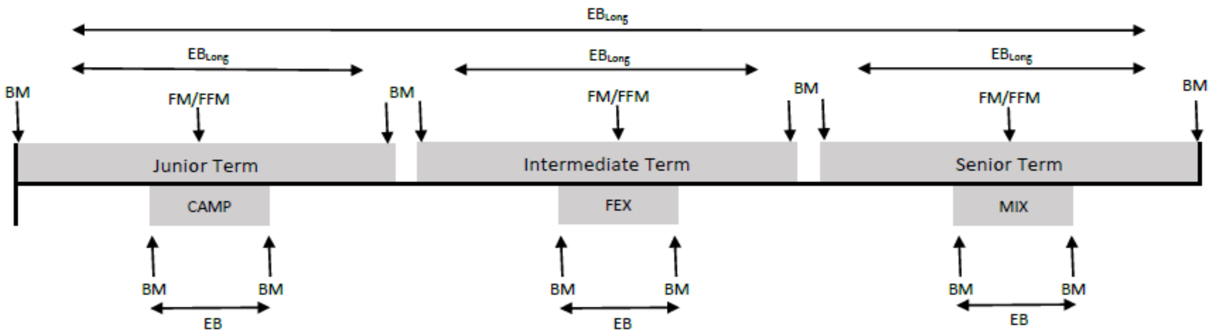

Figure 5.1: *Study design schematic of the 44-week Commission Course, describing the time points that Body Mass (BM), Fat Mass (FM) and Fat Free Mass (FFM) were taken to calculate acute Energy Balance (EB) during nine days during camp based training (CAMP), five days during field exercise (FEX) and nine days during combined camp and field training (MIX), and Longitudinal EB (EB_{Long}) over each term (Junior, Intermediate and Senior Term), and year*

5.3.3 *Anthropometry*

Height (SECA, Birmingham, UK) and body mass (Fitbit Aria, CA, USA) were measured at the beginning of training and at the beginning and end of each term, before lunch, wearing minimal clothing (shorts and t-shirt, with no shoes) where possible. For the acute data collection phases, body mass was measured at the beginning and end of the data capture period for CAMP (day 9), and when they arrived back into camp after exercise on FEX and MIX (FEX: day 6 and MIX: day 10) to determine changes in body mass. Fat mass was calculated from Total Body Water (TBW) determined from DLW as described previously by Schoeller et al. (1980) in Section 3.3.1 in Chapter 3, and FM was calculated as the difference between body mass and FFM (Wells & Fewtrell, 2006; Wishart, 2011).

A small study was conducted to determine the agreement between body composition data measured from Duel Energy X-ray Absorptiometry (DXA) and TBW methods (Appendix A). This was undertaken to determine whether TBW measured using DLW can be used as a valid measure of body composition within the scope of this thesis. The results demonstrated no differences in FM or FFM between methods and a moderate–to-strong correlation, exhibiting good validity between FM and FFM measured via TBW compared to DXA, and thus suggested that TBW could be used as a valid measure of body composition within the current study.

5.3.4 *Energy Expenditure*

Free-living EE was determined using the DLW technique over each data collection period. On the evening prior to the start of data collection, baseline urine samples were provided and collected into a 35 ml tube. Following the collection of urine, the participants drank a single bolus dose of hydrogen (deuterium ^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H_2{}^{18}O$). The amount of isotope given was calculated according to each participant's body mass to provide 150 - 180 mg per kg of body mass of ^{18}O and 50 - 80 mg per kg of body mass of ^{2}H. Isotopes were purchased, and analysed, by the Medical Research Council Elsie Widdowson Laboratory (MRC EWL). To ensure the whole dose of isotope was administered, the glass vials were refilled with additional water, then participants consumed the second bolus of water. Following administration of the DLW, urine samples were collected each day for 10 days, avoiding the first void of the day. Urine samples were aliquoted into 35 ml Eppendorf tubes and were refrigerated at 3 degrees until analysis. Urine samples were analysed at the MRC EWL by isotope ratio mass spectrometry to measure the ratios of ^{2}H / ^{1}H and ^{18}O / ^{16}O in the samples and to determine the rate of tracer decrease over the 10-day period. The determination of EE was completed using the multi-point method of Coward (1988) assuming a respiratory quotient of 0.85.

5.3.5 *Energy Intake*

Energy intake was measured via dietary weighing and food diaries as described in Section 4.3.4 in Chapter 4.

5.3.6 *Energy Balance*

Energy Balance was quantified over the three acute data collection periods during each term (CAMP, FEX, MIX) using Equation 5.1 (Hall et al., 2012), where EI was measured through dietary weighing, and EE was measured through DLW.

$$EB = EI - EE$$

Equation 5.1: *Calculation of Energy Balance (EB) through Energy Intake (EI) and Energy Expenditure (EE)*

Longitudinal EB over each term, as well as over the whole CC, were estimated using Equation 5.2.

$$Longitudinal\ EB = \frac{(9082 \times \Delta FM) + (1434 \times \Delta FFM)}{t}$$

Equation 5.2: *Calculation of longitudinal Energy Balance (EB) through the change (Δ) in Fat Mass (FFM) and Fat Free Mass (FFM) over time (t)*

Where ΔFM and ΔFFM is the change in FM and FFM, respectively, calculated through TBW and assuming an energy cost of 1434 kcal per kilogram of FFM, and 9082 kcal per kilogram of FM (Hall et al., 2011). *t* is the time period over which the body mass measures were taken.

5.3.7 *Data Analysis*

Two participants withdrew from FEX due to injury and a total of thirteen participants were used in the final data analysis. Results are expressed as mean ± one standard deviation. Estimated body mass from acute EB was calculated through the revised Forbes equation (Hall, 2008) which predict the energy density of men and women as 7167 kcal and 6689 kcal per 1 kg of body mass lost or gained, based on the body mass for individuals with less that 25kg of FM. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS; IBM SPSS version 23 for Windows, IBM Corporation, Chicago, IL). Statistical significance was set at $p < 0.05$. Descriptive statistics were used to assess assumptions of the data, and normality was confirmed using Shapiro-Wilk tests for dependent variables. Homogeneity of variances was confirmed using Levene's test. A repeated measures Analysis of Variance (ANOVA) with reported effect size (partial eta squared [η^2_p]) was used to compare body mass, FM and FFM over the year (Junior, Intermediate and Senior Term), as well as EB and actual body mass change over each condition (CAMP, FEX, MIX). Significant interactions were analysed with Bonferroni *post hoc* test to determine the location of the pairwise differences. Planned paired t-test with reported effect sizes (Cohens D) were used to determine the difference in body mass from the beginning to the end of each acute data collection period. Interpretation of Cohen's d is as follows: ≤ 0.2 trivial effect , 0.21 - 0.50 small effect, 0.51 - 0.80 moderate effect and ≥ 0.8 a large effect (Cohen, 1988). A Wilcoxon signed rank test, a non-parametric equivalent, was used to compare the mean difference in body mass from beginning to end of the acute period for CAMP. For each condition, actual body mass change and estimated body mass change were correlated, and the difference between each were compared with a one-sample t-test, where a result of no difference and a correlation would be further assessed for agreement.

5.4 Results

5.4.1 *Longitudinal Energy Balance*

Over the whole CC, body mass differed ($F_{(2.2,26.7)} = 5.26$, $p = 0.010$, $\eta^2_p = 0.305$), where a gain in body mass was seen from the beginning of Junior Term (75.1 ± 9.3 kg) to the beginning of Senior Term (77.6 ± 9.3 kg; p = 0.018, d = 0.268), as well as from the end of Junior Term (76.1 ± 8.9 kg) to the end of Senior Term (77.3 ± 9.4 kg; p = 0.039, d = 0.131), shown in Figure 5.2a. Despite no changes within each term, a large range was seen between participants (Junior Term: +5.2 to -5.2, Intermediate: +3.2 to -1.8, and Senior Term: +4.0 to -4.5 kg). Likewise, over the year, there was a large range between participants (+5.9 to -4.0 kg). Estimated longitudinal EB, via changes in body mass from the beginning to the end of each term, demonstrated that participants would have been in an overall positive EB during Junior Term (39 ± 132 $kcal \cdot d^{-1}$), Intermediate Term (30 ± 48 $kcal \cdot d^{-1}$) and Senior Term (22 ± 112 $kcal \cdot d^{-1}$). Over the year, due to a small increase in body mass (2.2 ± 3.3 kg), participants would appear to have been in an overall positive EB during the entirety of the CC (49 ± 75 $kcal \cdot d^{-1}$).

Once-termly measurements, showed a difference in FM ($F_{(2,24)} = 4.792$, $p = 0.018$, $\eta^2_p = 0.285$) and FFM ($F_{(2,24)} = 4.262$, $p = 0.026$, $\eta^2_p = 0.262$; Figure 5.2b), between terms, where participants gained FM from Intermediate Term (15.0 ± 4.3 kg) to Senior Term (18.3 ± 4.2 kg; p = 0.028, d = 0.776). There was no difference in FM from Junior (18.3 ± 3.6 kg) to Intermediate Term (p = 0.058, d = 0.832) nor Junior to Senior Term (p = 1.00, d = 0.00). Fat free mass increased from Junior Term (58.1 ± 10.3 kg) to Intermediate Term (61.3 ± 10.2 kg; p = 0.015, d = 0.312), however there was no difference in FFM between Intermediate and Senior Term (59.7 ± 9.1 kg; p = 0.467, d = 0.166), nor Junior to Senior Term (p = 0.703, d = 0.165).

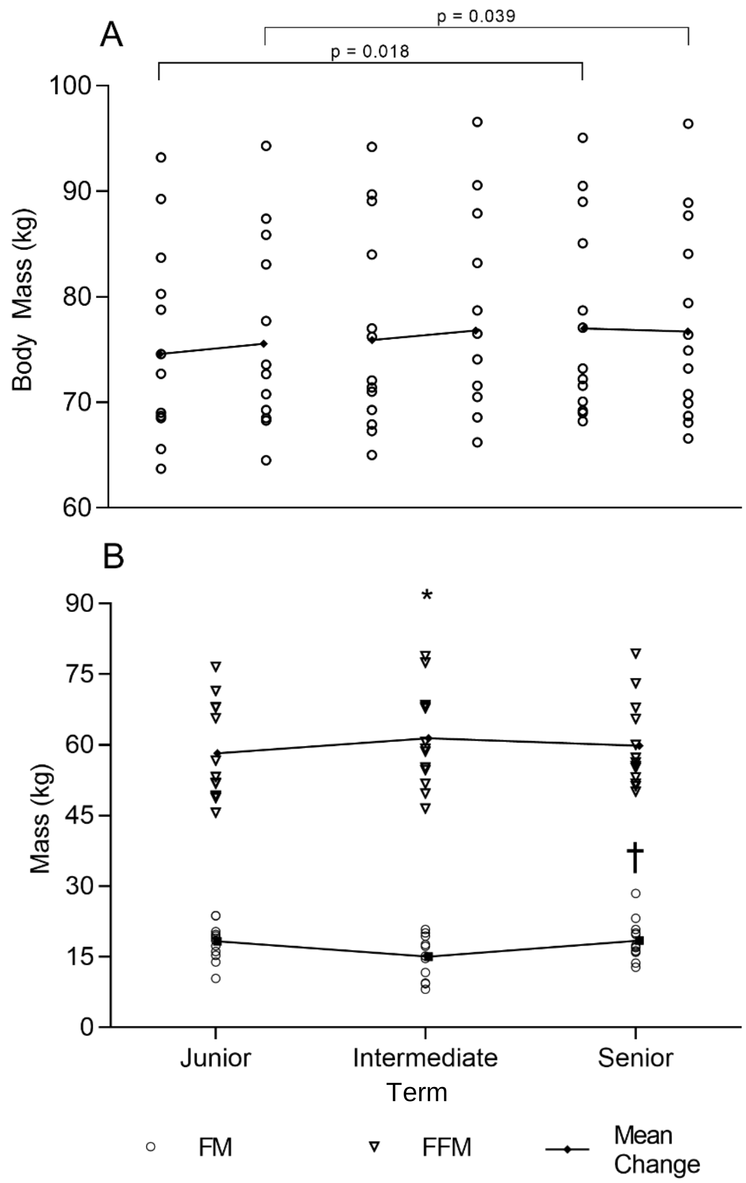

Figure 5.2: *Individual body mass (A), individual Fat Mass (FM; ○) and individual Fat Free mass (FFM; Δ; B) with average change (solid line) from the beginning to end of Junior, Intermediate and Senior Term. The bracket represents the difference in body mass between conditions, * represents a difference in FM or FFM from Junior Term; † represents a difference in FM or FFM from Intermediate Term, p < 0.05*

5.4.2 *Acute Energy balance*

Figure 5.3a shows that the average EB was different over condition ($F_{(2,24)} = 48.23$, $p < 0.001$, $\eta^2_p = 0.801$), where participants were in a greater negative EB in FEX compared to CAMP (mean difference [95% CIs]: 1497 [1020 - 1974], p < 0.001, d = 3.171) and MIX (909 [533 - 1285], p < 0.001, d = 2.408), as well as in greater negative EB in MIX compared to CAMP (588 [166 - 1010], p = 0.003, d = 1.402; Table 5.1).

Table 5.1: *Energy intake, expenditure and balance during acute periods of camp training (CAMP), field exercise (FEX) and combined camp and field training (MIX)*

	CAMP	FEX	MIX
Energy Intake ($kcal \cdot d^{-1}$)	3484 ± 848	2502 ± 790	3927 ± 619
Energy Expenditure ($kcal \cdot d^{-1}$)	4134 ± 609	4692 ± 776	2646 ± 484
Energy Balance ($kcal \cdot d^{-1}$)	-692 ± 506*	-2190 ± 436†	-1280 ± 309*†

* significant difference from CAMP, † significant difference from FEX, $p < 0.05$.

Based on EB during each conditions, estimated body mass change, calculated from the revised Forbes equation (Hall, 2008), was calculated to be -0.9 ± 0.6 kg on CAMP over nine days, -1.6 ± 0.3 kg on FEX over five days, and -1.7 ± 0.4 kg on MIX over nine days. Actual body mass change did not differ between conditions ($F_{(2,22)} = 2.836$, $p = 0.080$, $\eta^2_p = 0.205$; Figure 5.3b). Planned pairwise comparisons showed that body mass did not differ from the beginning to the end of data collection for CAMP (t (12) = -1.30, p = 0.219, d = 0.057), FEX (t (12) = 1.62, p = 0.131, d = 0.066) or MIX (t (12) = 0.79, p 0.445, d = 0.022). Actual and estimated body mass change (Figure 5.3c) were different from each other, and were not correlated on CAMP (p = 0.009; r = -0.037, respectively), FEX (p = 0.014; r = 0.450), or MIX (p < 0.001; r = -0. 026).

Figure 5.4 demonstrates total EB (average daily EB x duration) during CAMP (-6231 ± 4555 kcal), FEX (-10,984 ± 2273 kcal) and MIX (-11,560 ± 2898). Despite CAMP showing the smallest total EB, it had the greatest range between participants (-16,969 to 255 kcal) compared to FEX (-13,417 to -5190 kcal) and MIX (-15,558 to -5941 kcal), however no participant demonstrated a total EB above the threshold of -19,109 kcal to elicit a small change in performance.

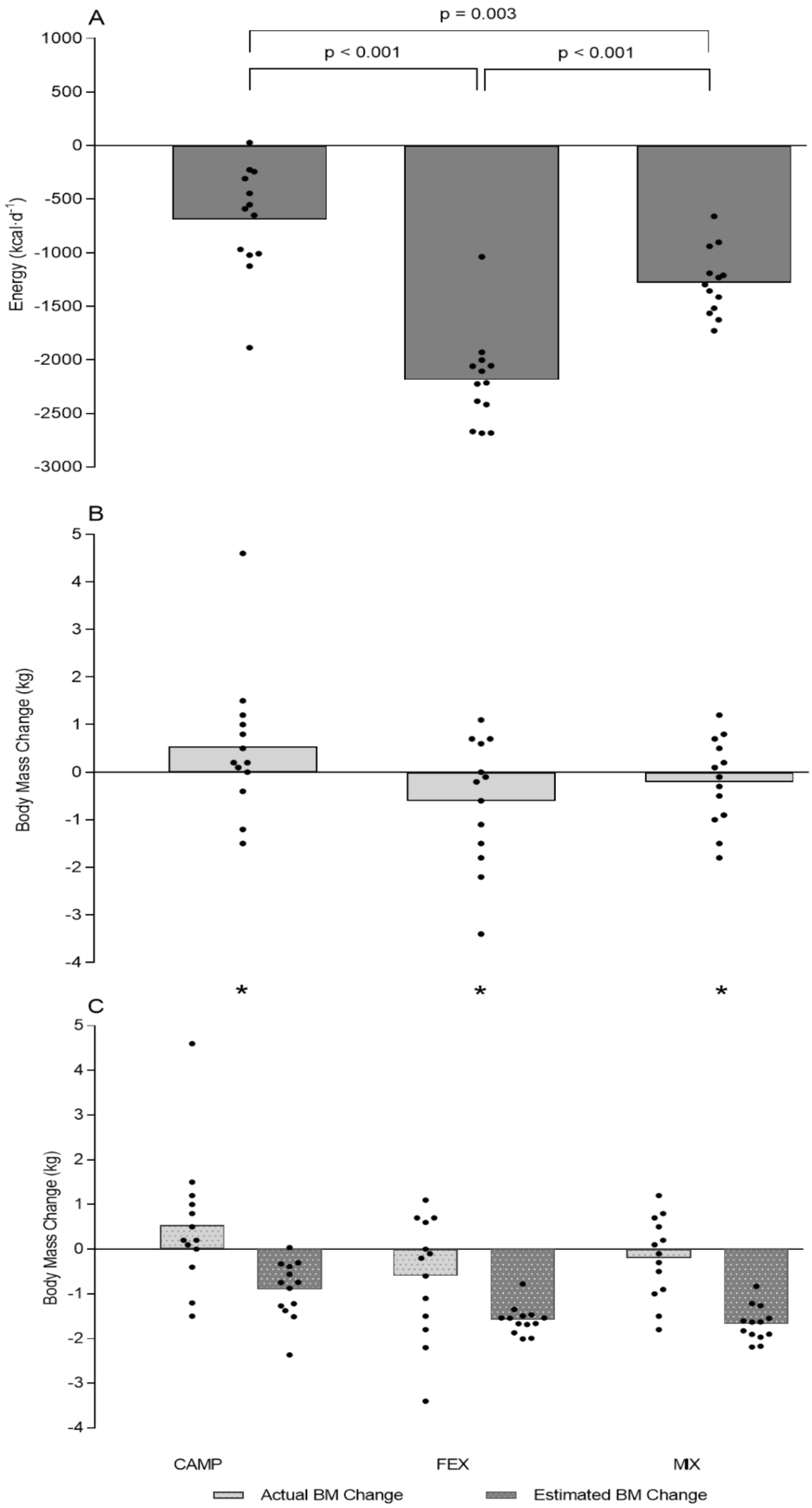

Figure 5.3: *Acute Energy Balance (EB; A), Body mass change (B), and difference in actual body mass and estimated body mass change (C), during camp training (CAMP), field exercise (FEX) and a combined camp and field based training (MIX). The bracket represents a difference between conditions, * represents a difference between estimated and actual body mass change, p < 0.05*

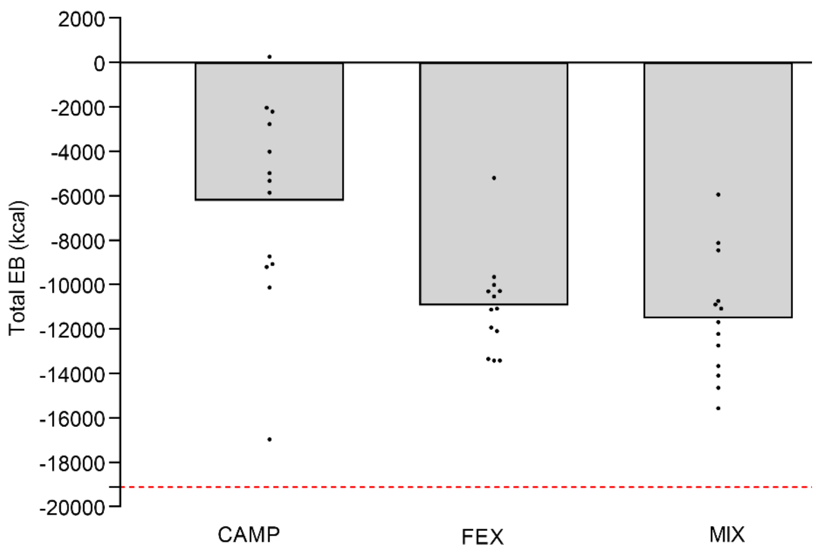

Figure 5.4: *Average and individual total Energy Balance (EB; average daily EB x duration) during camp training (CAMP), field exercise (FEX) and a combined camp and field based training (MIX)*

5.5 Discussion

The present study is the first to describe both longitudinal and acute EB during British Army OC training. The study quantified longitudinal EB calculated from the changes in body mass and composition over each term (3 x 14 weeks) as well as the whole of the CC (44 weeks). It also investigated acute EB calculated from EE and EI over three different training settings (CAMP: nine days, FEX: five days; MIX: nine days), as well as measuring body mass changes during the same period to determine the relationship between the measurements.

The main findings of the present study were that over each term, no overall mean body mass changes were seen, and that longitudinally, participants appeared to be in positive EB on average over the CC, despite the arduous nature of training. Similar to the present study, previous work during British Army Parachute Regiment training, a notoriously difficult 24-week course, showed that recruits were in an overall positive EB determined through an increase in body mass (2.6 ± 3.5 kg) (Wilkinson et al., 2008). Although the authors did not aim to quantify EB, they demonstrated similar EE to the present study during short 10-day periods (~4540 kcal·d^{-1}), as well as periods of extremely high EE (5970 kcal·d^{-1}) during the middle of the course. The positive EB over the course was likely due to recruits supplementing their diet with approximately 1200 kcal·d^{-1}, in addition to the 3200 kcal·d^{-1} that they were provided with. On shorter courses (8 - 12 weeks) in the British Army, it has been shown that recruits did not lose body mass from the beginning to the end of the course despite the progression of the intensity of training, demonstrating that the military training appeared to be within the capabilities of the recruits (Blacker et al., 2009; Richmond et al., 2012). Conversely, during an 8-week British Army Section Commanders' Battle Course, soldiers lost 5.1 ± 2.6 kg body mass (Richmond et al., 2014), equating to a deficit of approximately -644 $kcal \cdot d^{-1}$. However, it must be noted that the body mass loss reported was accounted for by a reduction in FM rather than FFM, and therefore was described as a positive adaption of the course.

In the present study, despite the overall gain in body mass over the year, a high degree of individual variation was observed where the greatest loss of body mass was 4 kg and the greatest gain was 6 kg, equating to the small overall difference. Large individual variations were also observed for the changes in FM and FFM, where the eight participants that gained body mass between the Junior and Intermediate Term tended to show an overall increase in FFM and decrease in FM. However, the seven participants who lost body mass during this time also tended to show an increase in FFM and decrease in FM. Between Intermediate and Senior Term, opposing results were observed in 12 participants who gained body mass, where those whose body mass increased tended to lose FFM and gain FM. The one participant whose body mass decreased also had a decrease in FFM and increase in FM. Therefore, although the average over the year demonstrated no significant change in body mass, individual variations much be taken into consideration. Siddall, Bilzon, et al. (2019) demonstrated that over 10 weeks of basic military training, body mass remained unchanged and fat mass deceased, whilst lower leg muscle density increased suggesting that gaining lean mass whilst decreasing fat mass is likely advantageous for military training. The individual variation in the present study demonstrated favourable changes between Junior and Intermediate Term and are therefore likely to be a reflection of positive training adaptations within the training course. Harwood et al. (1999) reported similar fluctuations in body mass, FM and FFM during the same CC, which is unsurprising given the structure of the course. The increase in body mass and FM between Intermediate and Senior Term can be interpreted in the context of the type of training during each term, where during Junior Term and Intermediate Term, OCs are prescribed a specific and structured training plan that focuses on fitness development. During this time, OCs are on a more rigid and structured training regime and are restricted from buying personal food items in the first six weeks of training. Therefore, it would be expected that FFM would increase and FM would decrease more in the first two thirds of the training course, which was demonstrated in the present study. During Senior Term, although training is still structured, OCs are allowed a more flexible regime, in which social and external events also occur more frequently and there is less emphasis on physical training. Although it cannot be speculated from the current data, it is suggested that with more frequent measurements of nutritional intake, body mass and composition measurements, that individual body mass responses to various types of training would be better explained, and thus would allow a greater indication of EB throughout training.

Although OCs appear to have been in a net stable EB during the course, it was clear that there are multiple training periods which elicited negative EB through the year. This is not uncommon for personnel, undertaking military training courses, to experience periods of high EE, due to the continuous nature of work and training consisting of intermittent periods of moderate to high intensity exercise, combined with periods of prolonged low intensity exercise (Henning et al., 2011; Skiller et al., 2005; Tharion et al., 2005; Wilkinson et al., 2008). This pattern is often seen in military training courses, where students can be physically active for 16 - 22 hours a day (Fairbrother et al., 1995; Hoyt et al., 2001; Tharion et al., 2004; Tharion et al., 2005). It was therefore unsurprising that the three data periods analysed in the present study demonstrated negative EB. Average EE during the data collection phases were similar to those previously reported during comparable training. Wilkinson et al. (2008) and Blacker et al. (2009) reported recruits' EE to be 4732 ± 700 $kcal{\cdot}d^{-1}$ and 3633 ± 359 $kcal{\cdot}d^{-1}$, respectively, and Richmond et al. (2014) demonstrated that during the Section Commanders' Battle Course soldiers expended up to 5094 ± 471 $kcal{\cdot}d^{-1}$ over 10 days using DLW. Between conditions, negative EB was modest during CAMP compared to FEX and MIX. During some short-term training exercises, it is not uncommon for EE to be exceptionally high and exceed the 4000 kcal that are provided within the supplied rations. Soldiers are also known to "field strip" their rations to increase available packing space and limit the amount of weight carried (Pasiakos & Margolis, 2017), choosing to keep specific items based on personal preference, but reducing their potential EI. During these times, EI will unlikely match EE. In the present study, the largest negative EB was observed during FEX, where participants were undertaking low-moderate continuous physical activity over a 5-day period, where they were also deprived of sleep, which is common during military training exercises (Margolis et al., 2013; Nindl et al., 2007). Margolis, Murphy, et al. (2014) found that Norwegian soldiers expended up to 6800 $kcal{\cdot}d^{-1}$ (measured via DLW) during a 3-day arctic military exercise, but ate approximately 3400 $kcal{\cdot}d^{-1}$ (measured via dietary weighing), equating to 50% of the energy required to maintain EB.

Although the CC, like other military training courses, are dispersed with periods of negative EB, it seems likely that personnel will match these times with periods of positive EB due to supplementing their diet with additional food. During training, EE and EI can be highly variable, resulting in energy deficits from day-to-day. Edholm and Fletcher (1955) showed that over 14 days of training, OC's EI was far from matching EE on a daily and weekly basis. On days where EE was high, EI was considerably lower, possibly due to lack of time to eat. However, this deficit was then corrected for in the subsequent two days, demonstrating that including recovery periods within examination of EB gives a more complete picture of that individual than looking solely at the exercising period. In the present study, although large energy deficits were seen within the acute periods, the small change in body mass measured longitudinally could indicate that participants, consciously or unconsciously, adjusted their intake after periods of large energy deficit. With the nature of military training, it is often the case that military training courses are designed to elicit military specific adaptations, used to better train and select appropriate personnel for mission requirements based on the scenario of extreme decrements. Therefore, the utility of both acute and longitudinal EB is important for the monitoring of soldiers to ensure that in times of short exercise periods, soldiers that are not adequately nourished in terms of total EI, are soon given adequate recovery from short-term negative EB, for optimal physical and cognitive performance, as well as ensuring long-term health and wellbeing.

Although it was not possible to perform more comprehensive physiological monitoring in the current study, an undesired negative EB has been found to adversely impact health, such as loss in bone mass over one sporting season (Carbuhn et al., 2010; Klesges et al., 1996), increase in injury rates (Dixon & Fricker, 1993; Schlabach, 1994), and compromised physical performance (Montain & Young, 2003), which has been attributed to body mass loss, potentially driven by associated reductions in FFM (Nindl et al., 2007). However, it is unclear what magnitude and duration of negative EB may elicit these effects. Previous studies have suggested that field exercises that are short (e.g. up to 3 days) and produce small decreases in body mass (< 3%), are primarily due to water losses or dehydration rather than FM / FFM, and would not be detrimental to performance in the short-term (Friedl, 1995), compared to longer exercises where there are greater losses in body mass (> 5%) (Alemany et al., 2008; Nindl et al., 2007). Margolis, Murphy, et al. (2014), who investigated EB using EE (measured via DLW) and EI (measured via food logs and wrapper discards) during a short-term 7-day arctic exercise, found that the soldiers experienced negative EB of 2900 $kcal \cdot d^{-1}$, and that lower-body peak power declined by ~330 W (7%) from baseline even though participants only lost 3% of initial body mass. Additionally, a meta-analysis by Murphy et al. (2018) demonstrated that the combination of daily EB and duration, expressed as total EB (daily EB X duration) was associated with decreases in both lower-body power and strength after military training operations. The authors suggested that a negative EB that participants could endure without eliciting any declines in performance was found to be -1166 $kcal \cdot d^{-1}$ or a total of -8162 kcal over the duration of the exercise / operation, and that a total negative EB should be limited to -5686 to -19,109 kcal for an entire operation (between 7 to 64 days), corresponding to a zero to small effect on performance, respectively. In the present study, participants were in an average net energy deficit of ~11,000 kcal in FEX and ~11,500 in MIX whereby no individual exceeded a total EB of -19,109 kcal over the entire data collection period which, according to Murphy and Colleagues' predictions, would likely have resulted in little (2%) to no decline in performance.

Plausible explanations for the lack of change in body mass observed in the current study are that the period in which participants were in an energy deficit were too small to elicit any noticeable body mass loss. It may also be possible that accurate measurements of body mass changes have been compromised by fluid retention (Stroud et al. 1993). Therefore, temporary fluid imbalance may have affected the results of the participant's final body weight measured in camp. However, a more plausible explanation to the differences in estimated and actual body mass change are likely to be explained by measurement errors. During the acute periods, body mass was not always measured at the same time of day and thereby not controlled for eating. This was especially the case during FEX, where body mass was not measured immediately after the participants arrived back into camp, and therefore participants may have had the opportunity to 're-feed' and rehydrate themselves prior to measurements, thus explaining the lack of body mass changes seen during this period. It is therefore apparent that more frequent body mass measurements over acute and longitudinal periods would be more likely to determine overall individual EB, and thus gain a greater understanding of individual variation.

It is also plausible that the difference in estimated and actual body mass changes, were due to an underestimation of EI. It has been found that food diaries can be underestimated up to 37% in comparison to methods such as DLW (Goris et al., 2000), which appears to be constant across population groups, with younger men and women underreporting by 18 - 23% (Livingstone et al., 1990; Martin et al., 1996; Seale & Rumpler, 1997), elderly subjects by 12 - 31% (Goran & Poehlman, 1992), lean women by 18% (Lissner et al., 1989), and athletes by 13 - 35% (Edwards et al., 1993; Westerterp et al., 1986). Underestimation can be attributed to user measurement error, such as conscious or sub-conscious exclusion of foods (Capling et al., 2017; Howat et al., 1994; Magkos & Yannakoulia, 2003), under- or over-estimation of portion sizes, which tend to be underestimated when portions are large and overestimated when portions are small (Beasley et al., 2005; Bingham, 1987; Goris et al., 2000). However, validation studies on self-reported food intake in military personnel who are supplied with ration packs, have demonstrated an agreement of > 95% with DLW (DeLany et al., 1989), which was suggested to be due to the participants having pre-supplied food, and therefore taking away the need for dietary recall. The authors also believe that the combination of wrapper collection and food weighing to measure EI as well as DLW employed in the present study offered the most practical solution for trying to ensure all dietary intake was captured across all sampling periods in a field-based trial.

Although it cannot be assured as to whether the mismatch in estimated body mass and actual body mass was due to measurement error or participant underreporting, it is likely that due to the acute periods of high EE, participants would still be in an energy deficit during short periods throughout the course. During these periods, a balance is required between the point where negative EB can be tolerated without any harmful consequences to health, and where personnel can have detrimental consequences from injury. Therefore, further investigation is warranted to elucidate the physiological consequences of repeated short-term bouts of energy deficit that is due to intense sustained physical activity (Margolis et al., 2013).

5.6 Conclusion

In conclusion, the present study is the first to concurrently document longitudinal and acute EB throughout the CC. Military training prepares personnel for deployment using simulated operational exercises which typically involves high EE, limited food and sleep, often resulting in decreased body mass. The overall demands of OC training appear to be sustainable based on the maintenance of body mass, where OCs appear to be able to offset periods of large energy deficits by interludes of positive EB, creating a maintained EB longitudinally. Methods of measuring EB, whether by body mass changes, or through direct measurement of EE and EI, are highly variable. Therefore, it is recommended that using both direct measurement of EE and EI and changes in body mass acutely and longitudinally during military training is the optimal approach to estimating EB and providing an evidence base for nutritional interventions.

Study 1 and Study 2 have demonstrated the high physical demands during the CC are not always met with the appropriate nutritional intake, leading to periods of negative EB especially during periods of FEX. It is known that EB can be achieved in an energy-deficient state, albeit with an accompanying suppression of physiological and metabolic processes. Therefore, assessing EB through changes in body mass, especially during times of arduous training, may not be an appropriate indicator of optimal physiological function. Study 3, will therefore explore the estimated Energy Availability (EA) during these periods of training.

5.7 Military Relevance and Recommendations

The research presented in this study will help inform researchers, practitioners and personnel on the importance of the methodological considerations when measuring energy balance both longitudinally and acutely. It is recommended based on the data presented, that direct methods (dietary weighing and direct measures of energy expenditure, *i.e.,* DLW) be employed, where possible, when exploring acute periods of energy balance and that body mass and composition measurements be used as a supplementary method. In the absence of research staff, military personnel should be aware of the risks of large negative energy balance, and therefore should adopt methods of measuring body mass and composition both acutely and longitudinally to estimate their energy balance. By understanding the risks associated with large negative energy balance, military personnel may be able to optimally adapt and recover from training as well as mitigate the risk of performance decrement and injury risk.

Chapter 6

Study 3

Energy Availability during British Army Officer Cadet Training

6.1 Abstract

Background: Optimal Energy Availability (EA) is when sufficient energy is available after Physical Activity Energy Expenditure (PAEE) to support all body functions that underpin health, adaptation to training, and optimal performance. Energy availability is expressed relative to Fat Free Mass (FFM) and can be categorised as reduced EA (30 - 45 kcal·kg $FFM^{-1}·d^{-1}$) or low EA (≤ 30 kcal·kg $FFM^{-1}·d^{-1}$). Low EA has been associated with impaired health and performance, through a reduction in Energy Intake (EI) and / or increased Energy Expenditure (EE). The aim of this study was to investigate EA of Officer Cadets (OCs) during different training settings. **Method:** Twenty-six (14 men and 12 women) OCs volunteered to undertake measurements of EI and EE during Camp training (CAMP), Field Exercise (FEX) and a combination of both camp and field training (MIX). In each setting, the Doubly Labelled Water (DLW) technique was used to estimate Total Daily Energy Expenditure (TDEE) and researcher-led dietary weighing was used to estimate EI. Physical Activity EE (PAEE) was calculated by subtracting each participant's estimated Basal Metabolic Rate (BMR) and Thermogenic Effects of Food (TEF) from their TDEE. Energy availability was calculated using the following equation; EA = (EI – PAEE) / FFM. A two-way Analysis of Variance (ANOVA) was used to compare all variables between conditions (CAMP, FEX, MIX) and sex. **Results:** There was no difference in EA between sex for any period ($p > 0.05$). Energy availability was higher during CAMP (mean ± SD; men: 20 ± 11 and women: 26 ± 11 kcal·kg $FFM^{-1}·d^{-1}$) compared to FEX (men: -1 ± 10 and women: -1 ± 14 kcal·kg $FFM^{-1}·d^{-1}$; $p < 0.001$), however there was no difference between CAMP and MIX (men: 10 ± 10 and women: 12 ± 6 kcal·kg $FFM^{-1}·d^{-1}$; $p > 0.05$). **Conclusion:** On average, participants were in low EA during all three periods, due to high PAEE and low EI. During military training, personnel often complete periods of intense exercise leading to high EE that they are unable to match with EI, resulting in insufficient EA. Identifying periods of reduced and low EA could inform strategies to improve health, training adaptations, and performance of military personnel.

6.2 Introduction

Energy balance has typically been the used in research and practice to quantify differences in Energy Expenditure (EE) and Energy Intake (EI) in relation to health and physical performance (Loucks et al., 2011). However, more recently, the concept of Energy Availability (EA) has become more prevalent within research, especially in athletes. Unlike EB, which refers to total EI verses total EE, EA is the difference between EI and EE during exercise (or physical activity) relative to Fat Free Mass (FFM), and represents the energy remaining for other metabolic processes to ensure optimal physiological function (Mountjoy et al., 2018; Ong & Brownlee, 2017).

Although the measurement of Energy Balance (EB) provides a useful construct to consider the overall regulation of human body mass, it also has practical limitations, particularly when utilised within an exercise physiology context. Daily EI and Total Daily Energy Expenditure (TDEE) are both complex and interrelated phenomena that are difficult to measure (Papageorgiou, Dolan, et al., 2017). In addition to the errors associated with measuring the core components of the EB equation, the model also assumes that all physiological systems are functioning at an optimal level, which may not necessarily be the case. It has been proposed that an energy-deficient organism may reduce basal metabolism in an attempt to restore balance, albeit with a suppression of non-immediately essential physiological functions (Stubbs et al., 2004; Wade et al., 1996).

In an athletic population, EA of at least 45 kcal·kg $FFM^{-1}·d^{-1}$ is recommended to maintain body mass (Loucks et al., 2011) and supply adequate energy for all physiological functions (Logue et al., 2017). Subsequently, reduced EA (30 - 45 kcal·kg $FFM^{-1}·d^{-1}$) has been associated with; increased risk of impaired physiological functions and physical performance (Loucks et al., 2011); increased risk of bone stress injuries in both men and women (Papageorgiou, Elliott-Sale, et al., 2017), as well as increased risk of menstrual disorder and infertility in women (Loucks et al., 2011), and reduced testosterone levels in men (Burke, Close, et al., 2018; Hackney, 2020). Low EA (≤ 30 kcal·kg $FFM^{-1}·d^{-1}$) reduces the amount of energy used for thermoregulation, growth, cellular maintenance and reproduction in favour of the more crucial physiological mechanisms that are necessary for survival, with implications for health and performance (Mountjoy et al., 2018). The adaptations of these metabolic and physiological functions therefore reduce TDEE to prevent weight loss and thus promote survival, which in doing so, will obtain a new steady state EB (Melin et al., 2019). Therefore, although an athlete's weight may be stable, it may be possible that physiological functions are impaired secondary to low EA.

Although studies have traditionally focussed on the risk of the adverse effects of low EA in women (Loucks et al., 2011), it is now recognised that men may be at a greater risk to low EA that previously thought (Burke, Close, et al., 2018). Additionally, studies conducted on combat sport athletes have reported EA to be as low as < 5 kcal·kg $FFM^{-1} \cdot d^{-1}$ in men trying to make weight. The magnitude and time course of weight loss experienced by combat athletes are often similar to those reported in military personal during simulated expeditions (Nindl et al., 1997), however EA, specifically, has not been measured in military personnel before.

Within the military setting, extremely high EE from a combination of stressors such as sleep deprivation, fatigue and extended periods of low-to-moderate physical activity, combined with low EI due to 'field stripping' ration packs and limited time to eat, presents an high risk of unintentional mismatch of EE and EI (Margolis et al., 2013; Nindl et al., 2007). Matching dietary EI to Physical Activity EE (PAEE), and thus maintaining optimal EA is a challenge for many physically active populations, including those in arduous occupational roles (Papageorgiou et al., 2018). Low EA can occur through a reduction in EI and / or increased exercise load, which is often seen within military field exercises (Margolis et al., 2013; Nindl et al., 2007). The importance of identifying low EA, with a view to protecting the health and performance of athletes is being increasingly recognised (Bronwen et al., 2017; Logue et al., 2017; Melin et al., 2015; Ong & Brownlee, 2017; Papageorgiou, Elliott-Sale, et al., 2017; Silva & Paiva, 2014), however, research in military personnel is limited. Therefore, the aim of this study is to investigate the EA of men and women Officer Cadets (OCs) undertaking the 44-week Commissioning Course (CC) at the Royal Military Academy Sandhurst (RMAS).

6.3 Methods

6.3.1 *Participants*

Twenty-six participants (14 men and 12 women) volunteered, where 14 participants (seven men and seven women) volunteered for all three conditions, an additional five participants (three men and two women) volunteered for two conditions and a further seven participants (four men and three women) volunteered for one condition. Participants were treated as independent samples between each condition. Ethical approval was granted by the Ministry of Defence Research Ethics Committee (protocol number 780/MoDREC/16). All participants were provided with a verbal and written description of the study and were provided with the opportunity to ask the researchers any questions before providing written informed consent.

6.3.2 *Study Design*

Data were collected over three separate weeks of the CC; during Camp training (CAMP), Field Exercise (FEX) and combination of both camp and field training (MIX), as described in Section 4.3.2 in Chapter 4.

6.3.3 *Anthropometry*

At the beginning of each data collection period, body mass (Fitbit Aria, CA, USA) was measured before lunch, wearing minimal clothing. Fat mass was calculated from Total Body Water (TBW) determined from Doubly Labelled Water (DLW) as described previously by Schoeller et al. (1980) and described in Section 3.3.1 in Chapter 3, and FFM was calculated as the difference between body mass and Fat Mass (FM) (Wells & Fewtrell, 2006; Wishart, 2011).

6.3.4 *Energy Expenditure*

Total daily EE was measured via DLW as described in Section 5.3.4 in Chapter 5.

6.3.5 *Energy Intake*

Energy intake was measured via dietary weighing and food diaries as described in Section 4.3.4 in Chapter 4.

6.3.6 *Calculations*

Average TDEE over the whole data collection period was calculated through DLW as described above. Physical activity EE was calculated from TDEE minus their estimated Basal Metabolic Rate (BMR)[2] (Henry, 2007) and TEF which was set at 10 % of EI. Energy availability was calculated by subtracting mean PAEE from the mean EI, relative to FFM (Loucks et al., 2011) shown in Equation 6.1.

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[2] Male BMR = (14.4 x body mass) + (313 x height) + 113; Female BMR = (10.4 x body mass) + (625 x height) – 282

$$EA = \frac{(EI - PAEE)}{FFM}$$

Equation 6.1: *Calculation of Energy Availability (EA) from Energy Intake (EI) minus Physical Activity Energy Expenditure (PAEE) relative to Fat Free Mass (FFM)*

6.3.7 *Data Analysis*

Participants were treated as independent groups. Three participants were excluded from FEX due to injury. Results were reported as mean ± standard deviation unless otherwise stated. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS; IBM SPSS version 23 for Windows, IBM Corporation, Chicago, IL) and statistical significance was set *a priori* at $p < 0.05$. Descriptive statistics were conducted and normality was confirmed using Shapiro-Wilk tests for dependent variables. Homogeneity of variances was confirmed using Levene's test. A two-way Analysis of Variance (ANOVA) with reported effect size (partial eta squared [η^2_p]) was used to compare all variables between conditions (CAMP, FEX, MIX) and sex. Significant interactions with reported effect sizes (Cohens D) were analysed with Bonferroni *post hoc* tests to determine the location of the differences. Interpretation of Cohen's d is as follows: ≤ 0.2 trivial effect , 0.21 - 0.50 small effect, 0.51 - 0.80 moderate effect and ≥ 0.8 a large effect (Cohen, 1988).

6.4 Results

6.4.1 *Participant Characteristics*

Body mass, height, body fat percentage and FFM are summarised in Table 6.1. There was a main effect of sex for all variables, irrespective of condition, where men were heavier ($F_{(1,51)} = 88.75$, $p < 0.001$, $\eta^2_p = 0.635$), taller ($F_{(1,51)} = 17.67$, $p < 0.001$, $\eta^2_p = 0.257$), had more FFM ($F_{(1,51)} = 149.72$, $p < 0.001$, $\eta^2_p = 0.746$) and a lower body fat percentage ($F_{(1,51)} = 57.22$, $p < 0.001$, $\eta^2_p = 0.529$) compared to women. For body fat percentage only, there was a main effect of condition ($F_{(2,51)} = 4.08$, $p = 0.023$, $\eta^2_p = 0.138$), irrespective of sex, where body fat percentage was lower in FEX compared to CAMP (mean difference [95% CIs]: 3.6 [0.5 - 6.8], $p = 0.021$, $d = 0.709$) only.

Table 6.1: *Characteristics (mean ± standard deviation), Total Daily Energy Expenditure (TDEE), Physical Activity Energy Expenditure (PAEE), Energy Intake (EI) and Energy Availability (EA) of all men and women during camp based training (CAMP), field based exercise (FEX), and combined camp and field training (MIX)*

	CAMP		**FEX**		**MIX**	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**
n	10	10	8	9	10	10
Height (m)	1.79 ± 0.08	1.67 ± 0.04*	1.80 ± 0.07	1.69 ± 0.04*	1.79 ± 0.06	1.68 ± 0.04*
Body mass (kg)	84.2 ± 6.4	69.8 ± 5.4*	83.7 ± 7.2	69.2 ± 5.1*	86.1 ± 6.6	71.7 ± 3.7*
Fat free mass (kg)	68.1 ± 5.7	49.0 ± 3.3*	71.8 ± 6.0	53.8 ± 4.5*	70.4 ± 8.8	52.5 ± 3.8*
Fat percentage (%)	19.0 ± 2.9	29.8 ± 2.2*	19.1 ± 3.2	22.5 ± 3.9*	17.4 ± 4.9	27.0 ± 5.5*
TDEE ($kcal \cdot d^{-1}$)	4629 ± 446	3594 ± 319	5326 ± 527	3926 ± 562	4415 ± 548	3503 ± 349
PAEE ($kcal \cdot d^{-1}$)	2385 ± 307	1820 ± 286	3300 ± 441	2233 ± 510	2343 ± 606	1772 ± 306
EI ($kcal \cdot d^{-1}$)	3938 ± 903	3185 ± 425	3207 ± 668	1864 ± 444	3197 ± 386	2390 ± 95
EA ($kcal \cdot kg\ FFM^{-1} \cdot d^{-1}$)	20 ± 11	26 ± 11	-1 ± 9	-1 ± 14	10 ± 10	12 ± 6

* significant difference between men and women, p < 0.05.

6.4.2 *Energy Expenditure*

For TDEE, there was a main effect of condition ($F_{(2,51)} = 11.18$, $p < 0.001$; $\eta^2_p = 0.305$; Figure 6.1A), irrespective of sex, where FEX was greater than CAMP (mean difference [95% CIs]: 537 [171 - 903], p = 0.003, d = -0.709) and MIX (690 [324 - 1056], p < 0.001, d = 0.913), however no difference was seen between CAMP and MIX (p = 0.891, d = 0.236). Men had a greater TDEE compared to women ($F_{(1,51)} = 81.33$, $p < 0.001$, $\eta^2_p = 0.615$), irrespective of condition. There was no interaction effect ($F_{(2,51)} = 1.11$, $p = 0.338$, $\eta^2_p = 0.042$).

For PAEE, there was a main effect of condition ($F_{(2,51)} = 17.12$, $p < 0.001$, $\eta^2_p = 0.402$; Figure 6.1B), irrespective of sex, where FEX was greater than CAMP (mean difference [95% CIs]: 687 [353 - 1022], p < 0.001, d = -1.237) and MIX (734 [399 - 1068], p < 0.001, d = 1.185), but there was no difference between CAMP and MIX (p = 1.00, d = 0.094). Men had a greater PAEE compared to women ($F_{(1,51)} = 41.39$, $p < 0.001$, $\eta^2_p = 0.448$), irrespective of condition and no interaction effects were detected ($F_{(2,51)} = 1.71$, $p = 0.191$, $\eta^2_p = 0.063$).

6.4.3 *Energy Intake*

For EI, there was a main effect of condition ($F_{(2,51)}$ = 9.41, p < 0.001, η^2_p = 0.270; Figure 6.1C), irrespective of sex, where intake during CAMP was greater than FEX (mean difference [95% CIs]: 682 [219 - 1145], p = 0.002, d = 0.857) and MIX (714 [270 - 1157], p < 0.001, d = 1.116). However, there was no difference between FEX and MIX (p = 1.00, d = 0.048). Men had a greater EI compared to women ($F_{(1,51)}$ = 26.61, p < 0.001, η^2_p = 0.343), irrespective of condition. There was no interaction effect ($F_{(2,51)}$ = 0.936, p = 0.399, η^2_p = 0.035).

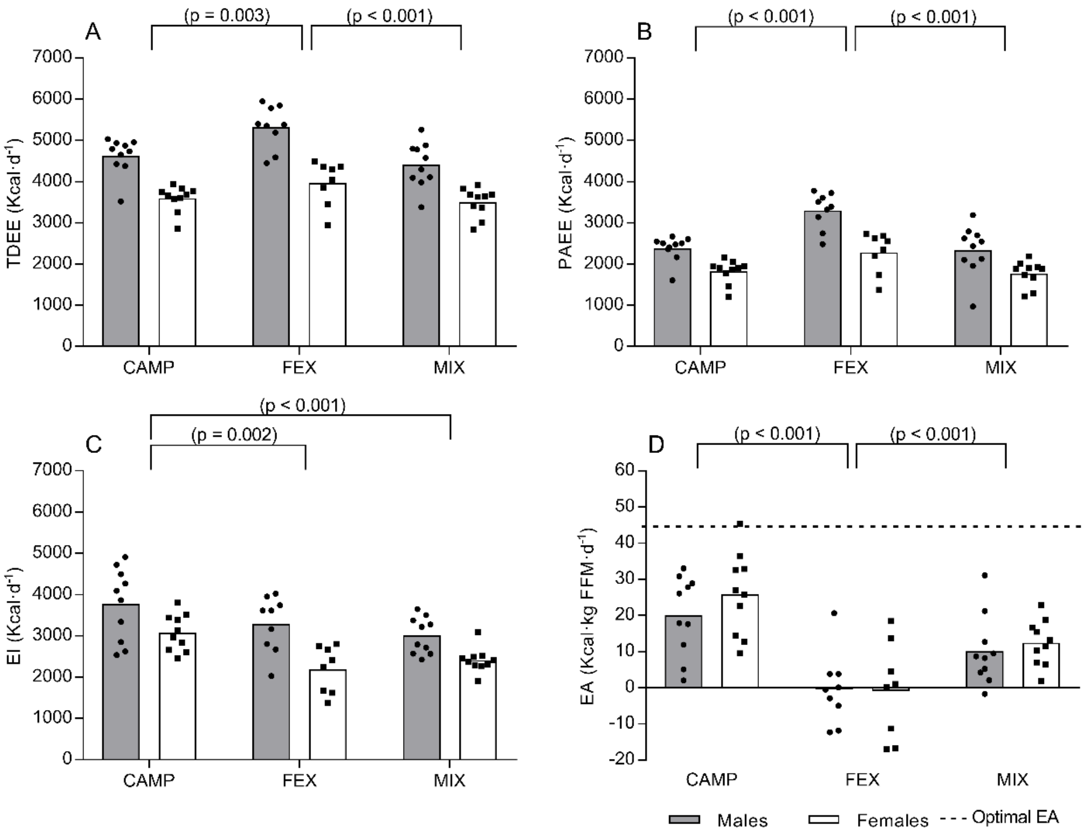

Figure 6.1: *Mean and individual data of men and women during camp training (CAMP), field training (FEX) and combined camp and field based training (MIX) for (A) Total Daily Energy Expenditure (TDEE; kcal·d^{-1}), (B) Exercise Energy Expenditure (PAEE; kcal·d^{-1}), (C) Energy Intake (EI; kcal·d^{-1}) and (D) Energy Availability (EA; kcal·kg FFM^{-1}·d^{-1}). The dashed line represents the optimal EA (45 kcal·kg FFM^{-1}·d^{-1})*

6.4.4 *Energy Availability*

For EA, there was a main effect of condition ($F_{(2,51)}$ = 21.65, p < 0.001; η^2_p = 0.459; Figure 6.1D), irrespective of sex, where EA during FEX was less than CAMP (mean difference [95% CIs]: 24 [15 - 32], p < 0.001, d = 2.103) and MIX (15 [7 - 25], p < 0.001, d = -1.401). However, there was no difference in EA between MIX and CAMP (p = 0.083, d = 0.700). There was no main effect for sex ($F_{(1,51)}$ = 3.06, p =

0.086, η^2_p = 0.057) or an interaction effect ($F_{(2,51)}$ = 1.03, p = 0.363, η^2_p = 0.039). During CAMP, one female participant was in optimal EA (Figure 6.2; 45 kcal·kg $FFM^{-1}\cdot d^{-1}$), five participants were in reduced EA (two men and three women; range: 31 - 36 kcal·kg $FFM^{-1}\cdot d^{-1}$) and 14 participants were in low EA (six men and eight women; range: 2 - 29 kcal·kg $FFM^{-1}\cdot d^{-1}$). In FEX, one female participant was in reduced EA (44 kcal·kg $FFM^{-1}\cdot d^{-1}$) and all other participants were in low EA (range: -17 - 21 kcal·kg $FFM^{-1}\cdot d^{-1}$), and in MIX one male participant was in reduced EA (31 kcal·kg $FFM^{-1}\cdot d^{-1}$), whereas all other participants were in low EA (range: -2 - 22 kcal·kg $FFM^{-1}\cdot d^{-1}$).

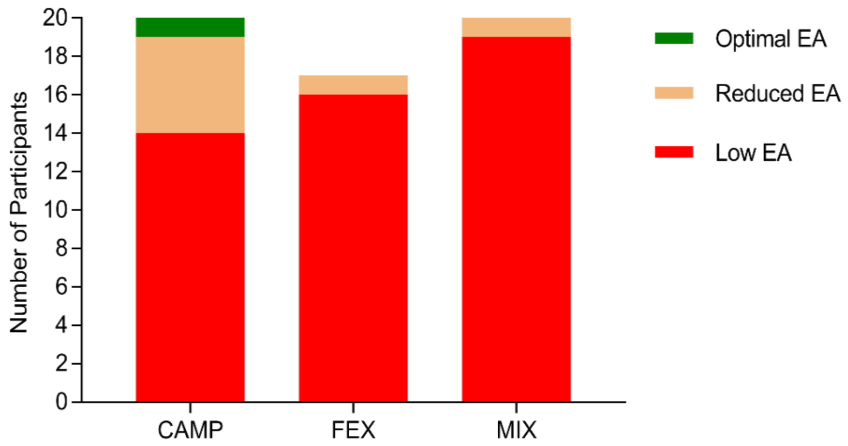

Figure 6.2: *Number of participants in optimal Energy Availability (EA; ≥ 45 kcal·kg $FFM^{-1}\cdot d^{-1}$), reduced EA (30 - 45 kcal·kg $FFM^{-1}\cdot d^{-1}$) and low EA (≤ 30 kcal·kg $FFM^{-1}\cdot d^{-1}$) for camp (CAMP), field based training (FEX) and combined camp and field training (MIX)*

6.5 Discussion

This is the first study to characterise EA in male and female OCs in three contextually-different military training settings. The key findings demonstrated that OCs were, on average, in low EA during all data collection periods, whereby EA in FEX was significantly lower than CAMP and MIX. The results suggest that the reduced and low EA, during both in-camp and field training, pose risk of compromised physiological function and physical performance (Loucks et al., 2011). High PAEE in the military results from work consisting of intermittent periods of moderate-to-high intensity and combined with periods of low intensity, prolonged movements (Henning et al., 2011; Skiller et al., 2005; Tharion et al., 2005; Wilkinson et al., 2008). McAdam et al. (2018) determined the EI and EE of OCs in the US Army using dietary logs and activity monitors. Their data suggested that average PAEE was high in comparison to other sporting studies, at approximately 1461 ± 286 kcal·kg $FFM^{-1}\cdot d^{-1}$. Although EA was not reported, when retrospectively estimated, it indicated that participants would have been in a low EA of approximately 17 kcal·kg $FFM^{-1}\cdot d^{-1}$, similar to that observed in OCs during CAMP and MIX in the present study. Similar to military training, high PAEE has been reported in endurance sports which have also reported reduced and low EA due to the high volume of training. Elite synchronised swimmers were shown to have EA as low as 18 ± 3 kcal·kg $FFM^{-1}·d^{-1}$ after four weeks of intense training where PAEE was found to be as high as 1230 ± 82 kcal·d^{-1} over 4 days (Schaal et al., 2017). Likewise, PAEE is extremely high in Tour de France cyclists who expended 3561 kcal·kg $FFM^{-1}·d^{-1}$ from exercise alone (Vogt et al., 2005). Although EA was not measured within the latter study, based on the EI and TDEE data presented, the athletes would have likely been in a predicted EA of ~6 kcal·kg $FFM^{-1}·d^{-1}$ over the 6 days. In the present study, during FEX, PAEE was demonstrated to be almost double (3300 kcal·d^{-1}) that reported in US Army recruits, and similar to that of the Tour de France cyclists, therefore it is unsurprising that the OCs were in a low EA.

As well as high PAEE, the reduced and low EA can be attributed to the low EI that was not matched to meet the demands of PAEE, especially in FEX. During some short-term training exercises, where personnel are provided with combat rations, similar to that of FEX, soldiers are known to "field strip" their rations to limit the amount of weight carried (Pasiakos & Margolis, 2017). This was demonstrated in Norwegian soldiers, participating in a 3-day arctic, who consumed only 54% of their supplied food (Margolis, Murphy, et al., 2014) despite TDEE being as high as 6800 kcal·d^{-1}. In the present study, although 4000 kcal·d^{-1} are provided within the rations, which was lower than reported TDEE, intake in men and women were 80% and 46%, respectively. However, this percentage does not take into account the additional food that OCs would have supplemented with, and therefore the percentage of the actual rations eaten would likely be lower. Therefore, whilst 4000 kcal is supplied, OCs would have needed to consume all food and beverages supplied in the rations along with an additional 2633 and 500 kcal·d^{-1}, for men and women, respectively, of supplementary energy to achieve optimal EA.

The present study demonstrated no sex differences in EA during any condition. Although studies of low EA in male athletes are few, it seems that the prevalence of low EA is found in similar sports as for women athletes (Mountjoy et al., 2014), including weight-sensitive sports, where leanness and / or body mass are important factors for performance or eligibility (*e.g.*, long-distance running, road cycling, boxing and wrestling) (Sundgot-Borgen et al., 2013). Only a few studies have investigated EA in the military directly, and not with the specific aim of comparing male and female soldiers. A study in male soldiers undertaking a combat course found that endocrine function decreased during a large energy deficit (1800 kcal·d^{-1}) (Gomez-Merino et al., 2002). Therefore, although low EA in men may involve different issues to women, such as reduced testosterone (Ackerman et al., 2018; Hackney, 2020), there is justification for concern that low EA may be prevalent and as problematic in men as it is in women. Owing to the earlier, relative, observation of the disruption of women's reproductive health from long-term intensive exercise training, there has been a greater volume of research in women on the potentially negative health consequences from energy deficit from athletic pursuits than in men (Burke, Close, et al., 2018; Hackney, 2020). The resultant collective term for the three key impacts (bone mineral density, eating disorder, infertility) in women is the Female Athletic Triad which, when coupled with impacts observed in both sexes, has culminated in the clinical term "Relative Energy Deficiency in Sports" (RED-S) (Mountjoy et al., 2014). The aforementioned emphasis has meant that the reference values for reduced- and low- EA have been determined from data in women, however, it is unclear if the reference values may differ from women, as men may be more metabolically robust against short-term energy reductions than women (Koehler et al., 2016). It is also important to note that although specific thresholds at which either physiological functions or performance can be impaired, there is likely to be a large degree of within and between participant variability (Williams et al., 2014). Due to the present study being observational, it is unknown to what degree men and women would have been affected by the consequences of low EA.

Although it has been hypothesised that persistent low EA could impair sport performance through a variety of different indirect mechanisms (*e.g.,* impaired recovery leading to premature reduction in physical, psychological, and mental capacity and impairment of optimal muscle mass and function), little is known currently about the time-frame and magnitude in which low EA can have a detrimental effect. Combat sports that are categorised by weight class often implement strategies to make weight, by manipulating body composition via techniques such as fluid restriction or fasting (Reale et al., 2017; Smith et al., 2001). Acute weight loss has been seen to exceed 10% of total body mass within 24 hours (Alderman et al., 2004; Crighton et al., 2016) resulting in a short period of low EA. In the present study, EI and EE were only collected for three short periods. Therefore, although participants were in a severely low EA during the FEX and MIX, it would be predicted based on the reduction in weekly PAEE during Intermediate and Senior Term (shown in Study 1), that EA may return to an optimal level when not on exercise.

Within military environments, where direct measures of EI and / or EE cannot be easily measured, clinical screening tools can be used for the detection low EA such as the low energy availability in females questionnaire (Melin et al., 2014), the RED-S clinical assessment tool (Mountjoy et al., 2018) or the cumulative risk assessment tool which have demonstrated to be validated methods to investigate the risk of low EA in athlete cohorts (Logue et al., 2019). Although these assessments include some degree of error, they have demonstrated to be a more cost effective and time effective tool to identify those who would benefit from a more detailed EA assessment (Burke, Lundy, et al., 2018), and thus may also be applicable in military personnel. Despite the recent abundance in studies investigating EA in athletes, there are still no clear guidelines on calculations of EA in a field environment, including the time-frame of the assessment and techniques used to measure each of the components of EA (Burke, Lundy, et al., 2018). Although DLW was used to measure TDEE, the measurement of PAEE has been shown to contribute a significant error to the calculation of EA (Burke, Lundy, et al., 2018) due to the lack of consensus on what constitutes exercise in a free-living environment, especially within the military where prolonged periods of moderate-to-light intensity exercise are often the contributing factors to the high TDEE (Margolis et al., 2013; Nindl et al., 2007). Additionally, the present study concentrated on measuring the variables for EI and EE to calculate estimated EA, and did not take any performance or health measures to understand the effects of reduced or low EA directly. Therefore, further research would be recommended to investigate EA within the field environment, such as carrying out analysis of indicators of hormonal function alongside measurements of Basal Metabolic Rate (BMR) to assess the physiological consequences of reduced or low EA.

6.6 Conclusion

The present study is the first to document EA in male and female OCs in the British Army. Energy availability was estimated to be low during all periods of military training due to high PAEE and extremely low EI, especially during times of field exercise training. There was no difference in EA between men and women, however it is unknown to what degree low EA may be prevalent and as problematic in men as it is women. Currently there are no clear guidelines on the specific measurements, or time and magnitude of EA and further research is warranted to explore whether an acute period of low EA would be detrimental to soldiers' health.

Studies 1, 2 and 3 have demonstrated that during training, OCs experience periods of large negative EB and reduced or low EA, accompanied by a suboptimal intake of carbohydrate and protein. During these periods, nutritional strategies such as the distribution of protein and carbohydrate around physical activity, may help mitigate the physiological consequences of energy deficit / low EA. However, the distribution of the intake of macronutrients during OC training has not previously been investigated. Therefore, Study 4, will explore the distribution of macronutrients throughout the day, to gain a greater understanding of the nutritional behaviours during training on camp and field exercises.

6.7 Military Relevance and Recommendations

The data presented in this study will help inform military personnel on the implications of a low energy availability and the potential detrimental effects on health and performance, such as decrements in physical performance and training time loss due to increased injury rates.

Military personnel should be aware of the risks of low energy availability and should take precautionary measures to ensure both themselves and their staff / students are mitigating these risks where possible. This should include:

- Awareness and education to all military personnel on the causes of low energy availability and the consequences that this may entail
- The use of screening techniques such as the ‘Low Energy Availability in Females Questionnaire’ described by Melin et al. (2014) to assess the risk of low energy availability
- Routine medical examinations of military personnel could include clinical evaluation of any energy deficiency’s and where possible be paired with biochemical, clinical and hormonal tests to attribute the findings of impaired function to low energy availability.

Chapter 7

Study 4

The Daily Distribution of Dietary Intake during British Army Officer Cadet Training

7.1 Abstract

Background: Timing of dietary energy and macronutrient intake around physical activity is important to optimise health and performance. During military training, personnel undertake repeated bouts of strenuous activity and are only able to eat when time permits. The aim of this study was to quantify the daily distribution of energy and macronutrient intake around physical activity during military training. **Method:** Twenty-six participants (14 men and 12 women) participated in three contextually-different periods of military training; during Camp training (CAMP), Field Exercise (FEX) and a combination of both camp and field training (MIX). Physical activity was measured continuously during each 10-day block using a wrist-worn tri-axial accelerometer from which Energy Expenditure (EE) was estimated. Dietary intake was measured via researcher led dietary weighing alongside food diaries, and dietary intake was categorised into meals (M1, M2, M3) and snacks (S1, S2, S3, S4). A one-way independent Analysis of Variance (ANOVA) was used to identify differences between conditions (CAMP, FEX, MIX) in body mass and height, as well as to compare total Energy Intake (EI), carbohydrate, protein and fat intake across conditions. **Results:** Hourly EE was lower during the typical sleep hours during CAMP and MIX, whilst FEX had consistently high EE throughout the day. During both CAMP and MIX, the intake of energy, carbohydrate and protein was greater at mealtimes compared to snacks ($p < 0.001$). During FEX there were no differences in mean energy and macronutrient intake across meal and snack times ($p > 0.05$), due to varied distribution of dietary intake between individuals. **Conclusion:** This variable distribution dietary intake during particularly arduous training may be suboptimal for sustaining occupational performance and recovering from physical activity. This is the first study to concurrently document the daily distribution of dietary intake and physical activity during military training. The results suggest that distribution of macronutrient intake could be adapted to support recovery from physical activity and enhance performance.

7.2 Introduction

Dietary intake of protein and carbohydrate after exercise enhances health and performance outcomes (Kerksick et al., 2008). As demonstrated in studies 1 and 2, during military training personnel undertake a variety of concurrent and repeated strenuous activities over a number of days and are only able to eat when time / situation permits (Blacker et al., 2009; Richmond et al., 2014). During these periods, nutritional intake often does not meet the required demands, and thus results in bouts of negative Energy Balance (EB; Study 2). Adequate provision and timing of dietary intake is critical during military training to maintain or improve physical and mental performance, enhance recovery and promote adaptation to training (Beals et al., 2015). However, unlike other sports and athletic settings, the varied nature of occupational demand and training means that optimal energy and macronutrient intake of military personnel is not clear, and therefore athletic nutritional guidelines may not be appropriate.

The timing of macronutrient intake, particularly protein, and its subsequent effects on training adaptations, has been explored in both controlled laboratory and sports performance settings, where typically macronutrient intake is manipulated around a single bout of exercise to determine the influence on performance, Muscle Protein Synthesis (MPS) or muscle damage (Areta et al., 2013; Nosaka et al., 2006; Roy et al., 2000). Typically, consuming protein immediately after resistance exercise, with an even distribution of intake throughout the day, is considered the most effective method for stimulating MPS compared to an excessively high protein intake at single and / or infrequent time points each day (Mamerow et al., 2014). Moreover, carbohydrate intake prior to, and during, exercise improves exercise performance at a given workload (Jeukendrup et al., 1997) and, when taken after exercise in combination with protein, promotes greater glycogen uptake and resynthesis (Ivy et al., 2002).

Whilst military training is not solely focused on resistance or endurance exercise performance, the application of these principles may be useful in the maintenance of muscle mass, protection against glycogen depletion and for optimising exercise recovery. Given the nature of military training, with its high daily physical activity levels over several weeks (Blacker et al., 2009; Richmond et al., 2014), nutritional requirements may differ from typical laboratory models of dietary intake timing around acute exercise. High fat intake has also been observed in US Special Forces (Tharion et al., 2004), which may occur when Energy Expenditure (EE) is high but time to eat is restricted, such as on operational exercise. The higher energy density of fat compared to carbohydrate or protein during this time may protect against large energy deficits. In addition, nutritional intake in military environments may also vary considerably between time training in camp and on field exercises. The aim of this study was to determine the daily distribution of dietary intake and energy expenditure during three different military settings; on camp only, during field exercise and a mixture of both camp and field exercise.

7.3 Method

7.3.1 *Participants*

Twenty-six British Army Officer Cadets (OCs; 14 men and 12 women) undertaking the 44-week Commissioning Course (CC) at the Royal Military Academy Sandhurst (RMAS) volunteered to participate in this study, where 14 participants (seven men and seven women) volunteered for all three conditions, an additional five participants (three men and two women) volunteered for two conditions and a further seven participants (four men and three women) volunteered one condition. Participants were treated as independent samples between each condition. Ethical approval was granted by the Ministry of Defence Research Ethics Committee (protocol number 780/MoDREC/16). As interaction effect of Energy Intake (EI) and condition was seen between sex in Study 1, which quantified that there was no difference in macronutrient intake between sexes when accounting for body mass, men and women were grouped together in the present study. Participants were provided with a verbal and written brief on the requirements of the study and offered the opportunity to ask questions before providing informed written consent.

7.3.2 *Study Design*

Data were collected during three separate blocks of time during the course; during Camp training (CAMP), Field Exercise (FEX) and MIX, as described in Section 4.3.2 in Chapter 4.

7.3.3 *Dietary Intake*

Energy intake was measured via dietary weighing and food diaries as described in Section 4.3.4 in Chapter 4. Daily distribution of energy and macronutrient intake was categorised into meals [M: breakfast (M1: 0600 - 0800), lunch (M2: 1200 - 1400) and dinner (M3: 1800 - 2000)], and snacks [S: pre-breakfast (S1: 0000 - 0600), mid-morning (S2: 0800 - 1200), mid-afternoon (S3: 1400 - 1800) and evening (S4: 2000 - 0000)] (Figure 7.1). Food items that were recorded as being eaten at the crossover point of the two categories, *e.g.,* 0800; M1 and S2, were classed as a snack or meal based on the nature of the item.

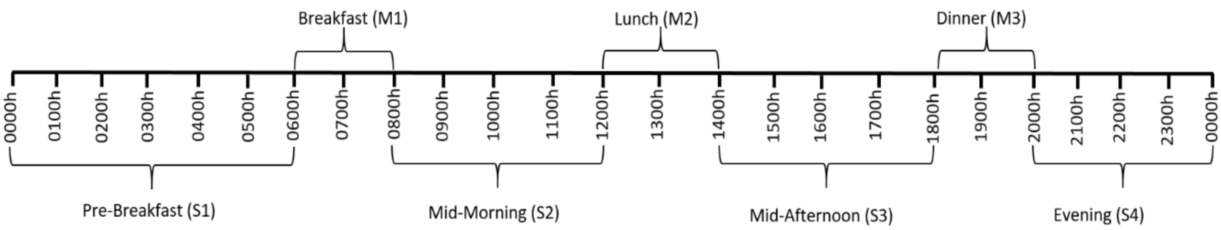

Figure 7.1: *Schematic of timings for Meal (M) and Snack (S) categories*

7.3.4 *Activity Monitoring*

Hourly EE over the training course was estimated using a wrist-worn tri-axial accelerometer as described in Section 4.3.3 in Chapter 4.

7.3.5 *Data Analysis*

Participants were treated as independent groups. Three participants were excluded from FEX due to injury. Results are reported as mean ± standard deviation unless otherwise stated. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS; IBM SPSS version 23 for Windows, IBM Corporation, Chicago, IL) and statistical significance was set *a priori* at $p < 0.05$. Normality was confirmed using Shapiro-Wilk tests for dependent variables. A one-way independent Analysis of Variance (ANOVA) with reported effect size (partial eta squared [η^2_p]) were used to identify differences between conditions (CAMP, FEX, MIX) in body mass and height, as well as to compare total EI, carbohydrate, protein and fat intake across conditions. If data were found to be non-normally distributed, Kruskal-Wallis tests were used to identify differences, and *post hoc* analysis were used to compare any significant pairwise effects. Paired sample t-tests with reported effect size (Cohens D) were used to compare the difference in average intake of energy, carbohydrate, protein and fat during meal times compared to snack times. Interpretation of Cohen's d is as follows: ≤ 0.2 trivial effect , 0.21 - 0.50 small effect, 0.51 - 0.80 moderate effect and ≥ 0.8 a large effect (Cohen, 1988).

7.4 Results

7.4.1 *Participant characteristics*

Participant characteristics are described in Table 7.1, where there was no difference in age ($F_{(2,54)}$ = 2.42, p = 0.099, η^2_p = 0.082), body mass ($F_{(2,54)}$ = 0.32, p = 0.725, η^2_p = 0.012) or height ($F_{(2,54)}$ = 0.15, p = 0.866, η^2_p = 0.005) between conditions.

Total EI was different between conditions (χ^2 (2) = 11.52, p = 0.003, η^2_p = 0.203; Table 7.1), where EI was greater during CAMP than FEX (mean difference [95% CIs]: 723 [162 - 1283], p = 0.009, d = 0.901) and MIX (710 [173 - 1247], p = 0.007, d = 1.107), however there was no difference between FEX and MIX (p = 0.100, d = -0.019).

7.4.2 *Macronutrient Intake*

Carbohydrate intake was different between conditions (χ^2 (2) = 9.23, p = 0.010, η^2_p = 0.175; Table 7.1), where carbohydrate intake was greater in CAMP compared to MIX (mean difference [95% CIs]: 101 [30 - 171], p < 0.003, d = 1.201). However, there was no difference between CAMP and FEX (p = 0.179, d = 0.558), or FEX and MIX (p = 0.476, d = 0.527). Protein intake was different between conditions ($F_{(2,54)}$ = 19.10, p < 0.001, η^2_p = 0.414), where participants had a greater intake of protein during CAMP compared to both FEX (49 [30 - 68], p < 0.001, d = 1.828) and MIX (27 [9 - 46], p = 0.003, d = 1.11), as well as protein intake being higher during MIX compared to FEX (22 [3 - 41], p = 0.024, d = 1.046). Fat intake was different between conditions (χ^2 (2) = 8.141, p = 0.017, η^2_p = 0.176; Table 7.1), were fat intake was higher in CAMP compared to FEX (29 [4 - 53], p = 0.019, d = 0.816), however there was no difference between CAMP and MIX (p = 0.088, d = 0.804) or FEX and MIX (p = 1.00, d = -0.238).

7.4.3 *Distribution of Dietary Intake*

The average EI of meal and snack times was different during CAMP and MIX but not during FEX, where mean difference is reported in Table 7.2. These results were mirrored when split by macronutrients, where during CAMP and MIX, average carbohydrate, protein and fat intake during meals were higher compared to snacks (Figure 7.2). However, there was no difference between the average intake of carbohydrates, protein or fat between meal and snack times during FEX.

Table 7.1: *Participant characteristics, dietary intake and energy expenditure during camp (CAMP), field exercise (FEX) and combined camp and field training (MIX)*

Variables	**CAMP**	**FEX**	**MIX**
n	20	17	20
Age (years)	23 ± 2	24 ± 2	24 ± 3
Height (m)	1.73 ± 0.08	1.74 ± 0.08	1.74 ± 0.08
Body mass (kg)	77.0 ± 9.3	76.6 ± 9.9	78.9 ± 9.0
Energy intake ($kcal \cdot d^{-1}$)	3420 ± 771†	2697 ± 837*	2709 ± 478*
Carbohydrate intake ($g \cdot d^{-1}$)	412 ± 101 (48 ± 4%)	356 ± 111 (53 ± 5%)	311 ± 63* (46 ± 1%)
Protein intake ($g \cdot d^{-1}$)	128 ± 29† (15 ± 2%)	79 ± 24* (12 ± 1%)	101 ± 18†* (15 ± 1%)
Fat intake ($g \cdot d^{-1}$)	134 ± 33† (35 ± 3%)	105 ± 38* (35 ± 4%)	112 ± 20 (37 ± 3%)

Data are presented as (mean ± SD) * significant difference from CAMP, † significant difference from FEX, $p < 0.05$.

7.4.4 *Total Energy Intake*

Distribution of average and individual protein (Figure 7.3, panels A - C) and carbohydrate (Figure 7.3, panels D - F) intake for each meal period is shown in Figure 7.3 alongside hourly EE. Group-average data demonstrates protein and carbohydrate intake in CAMP and MIX are distributed around standard core mealtimes but is more even during FEX. However, individual data shows that the assumed even distribution in FEX is a product of high inter-individual variation in intake pattern within this setting, resulting in similar values across core meal and snack periods. No data are shown during S1 for CAMP as no food was consumed during this period.

7.4.5 *Energy Expenditure*

Average hourly EE across the day is shown in Figure 7.3 (Panels G-I), demonstrating that during FEX, the group-average distribution of EE remained consistently high throughout the entire day (*i.e.,* no clear sleep / wake periods) compared to CAMP and MIX.

Table 7.2: *Statistical output from paired-sample t-tests with reported effect size and 95% Confidence Intervals (95% CIs) of energy and macronutrient intake between meal and snack times during camp training (CAMP), field exercise (FEX) and combined camp and field training (MIX)*

								95% CI for Cohen's d	
		Meal	**Snack**	**t**	**df**	**p**	**Cohen's D**	**Lower**	**Upper**
CAMP	Energy (kcal·d^{-1})*	869 ± 162	438 ± 152	11.21	19	< 0.001	2.51	1.59	3.40
	Carbohydrate (g·d^{-1})*	89 ± 18	43 ± 17	10.73	19	< 0.001	2.40	1.52	3.27
	Protein (g·d^{-1})*	32 ± 7	8 ± 4	17.31	19	<0.001	3.87	2.57	5.16
	Fat (g·d^{-1})*	28 ± 6	15 ± 5	10.5	19	0.001	2.35	1.48	3.20
FEX	Energy (kcal·d^{-1})	564 ± 283	562 ± 150	0.035	16	0.973	0.01	-0.47	0.48
	Carbohydrate (g·d^{-1})	70 ± 28	77 ± 20	0.98	16	0.344	0.24	-0.72	0.25
	Protein (g·d^{-1})	18 ± 11	17 ± 5	0.724	16	0.480	0.18	-0.31	0.65
	Fat (g·d^{-1})	25 ± 15	21 ± 7	1.26	16	0.225	0.31	-1.19	0.79
MIX	Energy (kcal·d^{-1})*	562 ± 89	203 ± 128	10.94	19	< 0.001	2.45	1.55	3.32
	Carbohydrate (g·d^{-1})*	55 ± 9	22 ± 13	9.851	19	< 0.001	2.20	1.37	3.01
	Protein (g·d^{-1})*	20 ± 4	4 ± 2	19.38	19	< 0.001	4.33	2.89	5.76
	Fat (g·d^{-1})*	21 ± 4	6 ± 3	13.38	19	< 0.001	2.99	1.94	4.02

* represents difference between intake at meal times and intake and snack times, p < 0.05.

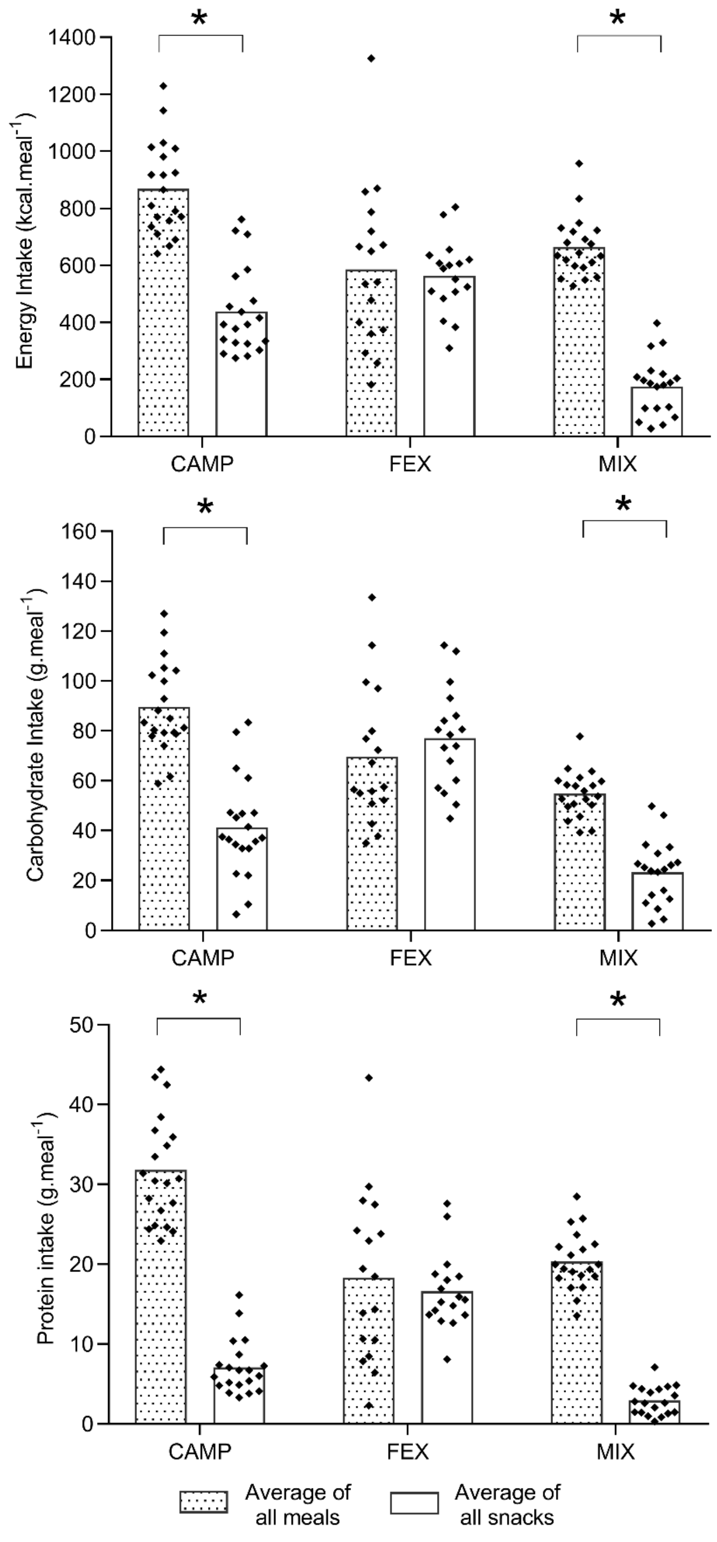

Figure 7.2: *Energy (kcal·meal^{-1}), carbohydrate and protein (g·meal^{-1}) of Meals (M) and Snacks (S) during camp training (CAMP), field exercise (FEX) and combined camp and field training (MIX) showing individual intakes.* represents statistical significance between meal and snack times, $p < 0.05$*

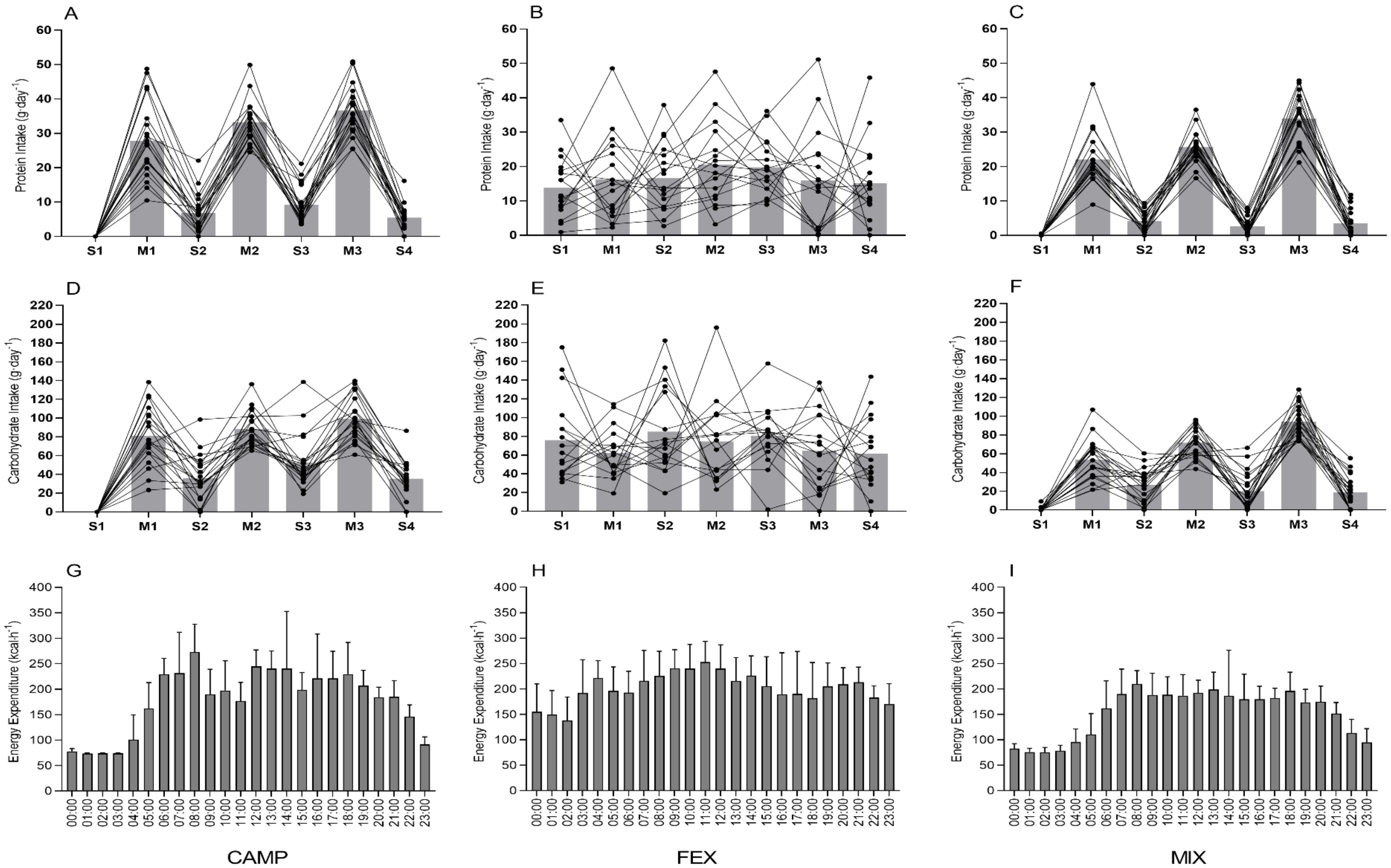

Figure 7.3: *Average and individual daily distribution of protein (A-C) and carbohydrate intake (D-F), and the distribution of estimated hourly Energy Expenditure (G-I) for training in camp (CAMP) on field exercise (FEX) and combined camp and field training (MIX)*

7.5 Discussion

This is the first study to characterise the daily distribution of energy and macronutrient intake in three contextually-different military training settings in relation to physical activity. The main finding was that during training settings on military camp, EI distribution centred around the expected three core meal times (breakfast, lunch, dinner). However, during a particularly arduous field-training exercise, which placed OCs in a severe energy deficit, distribution of EI contained high inter-individual variation, which was masked by a misleadingly "even" distribution when observing only group-average data. Based on the overarching principle that evenly distributing EI throughout the day is likely to promote maintenance of muscle mass and exercise recovery, individual data indicates that nutritional intake during arduous field training may be suboptimal for occupational performance.

Military training courses are designed, predominantly, to achieve military-specific aims which include promoting physical readiness for occupational performance but also training professional and technical skills of survival, leadership and soldiering. As such, strenuous field training that includes sleep disruption and elicits severe energy deficit is often designed deliberately to prepare personnel for the possible physiological consequences of deployment, despite being at odds with optimal strategies for long-term improvement in physical performance (Nindl et al., 2007; Richmond et al., 2014). During short-term moderate energy deficits, a combination of protein and carbohydrate intake at the higher end of the recommended athletic guidelines (6 - 10 of carbohydrate and 1.2 - 2.0 $g{\cdot}kg{\cdot}d^{-1}$ from protein) (Phillips & Van Loon, 2011; Rodriguez, 2013; Rodriguez et al., 2009) may support the demands of physical and tactical training, whilst also mitigating negative effects produced through nutritional deficits such as loss in lean body mass (Tarnopolsky, 2004). Study 1 demonstrated that the relative intake of carbohydrate and protein during CAMP (carbohydrate: 5.5 ± 1.5 and protein 1.7 ± 0.4 $g{\cdot}kg{\cdot}d^{-1}$) were in line the recommendations. However, during FEX and MIX, whilst fat intake remained similar to other conditions and on par with recommendations, carbohydrate (FEX: 4.5 ± 2.0 and MIX: 4.0 ± 1.2 $g{\cdot}kg{\cdot}d^{-1}$) and protein (FEX: 1.0 ± 0.6 and MIX: 1.3 ± 0.3 $g{\cdot}kg{\cdot}d^{-1}$) intake was below the recommended range.

At low intensity exercise, fat is the dominant energy source for metabolism and, being more energy dense than protein and carbohydrate, reduces the volume of food required to sustain high overall EE. Tharion et al. (2004) found that US Special Forces soldiers consumed protein in line with guidelines (14%) and carbohydrate slightly under recommended levels (48 %), while fat intake was substantially higher (35%). These findings suggest that choice or availability of food for OCs favoured high-carbohydrate or high-fat items during field exercise. Given participants in the current study were seen to be in a large energy deficit, achieving an EI to meet their EE may be unrealistic or impractical. However, it may be that replacing some carbohydrate-rich foods with items of higher fat content, or by consuming a higher proportion of fat overall, would have better protected against energy deficit than consuming more protein or carbohydrate.

During FEX, participants were provided with ration packs, which include three main meals, various snack products and drink mixes (*i.e.,* coffee, tea). Anecdotally, it was reported that items are often discarded uneaten by the soldier prior to exercise and are replaced with high sugar substitutes, which is reflected in the individualised peaks and high overall carbohydrate intake observed in FEX. Arguably, these findings are expected given the nature of field exercise where there are often no clearly defined periods of sleep, and OCs eat only when the context of their exercise objective permits, where high sugar foods may feel like a beneficial intervention against fatigue.

During CAMP and MIX, individual EI data more closely reflected the group means and demonstrated a more typical pattern of feeding around core meals, as well as demonstrating a typical wake / sleep cycle, with EE lower during the morning and evening. However, during FEX total daily EE was consistently high, and feeding patterns revealed no consistent distribution of energy or macronutrient intake, with participants' intake peaking at different times throughout each day. Given the consistently high EE on field exercise, it is likely that EI, protein and carbohydrate intake during FEX was suboptimal in meeting the acute demands of physical activity combined with restricted sleep and post-exercise recovery during the day.

The effects of optimising the timing of carbohydrate intake around exercise on performance is well-documented (Jeukendrup, 2014), but the distribution of carbohydrate intake around multiple daily exercise bouts in military training has not previously been examined. Prolonged exercise of moderate-to-high intensity will deplete carbohydrate stores, potentially leading to a decrease in work output (Coyle et al., 1985), muscle tissue breakdown and immunosuppression (Gleeson et al., 2004). The intake of carbohydrate pre-, during and post-exercise can offset these changes (Kerksick et al., 2008) and are important for exercise performance.

During the more structured settings in the present study (CAMP and MIX), the distribution of protein was similar to that reported for civilian adults and athletes, where daily peak protein (and energy) intake is skewed towards the evening meal. Specifically, protein intake during the evening meal is typically threefold greater compared with breakfast (evening; 38 g vs. breakfast; 13 g) in civilians (Mathias et al., 2017) and twofold greater (breakfast ~19 g; lunch ~25 g; dinner ~38 g) in athletes (Gillen et al., 2017). Mamerow et al. (2014) demonstrated that consuming a moderate amount (~30

g) of high-quality protein three times a day resulted in 25% greater MPS than the common practice of skewing the majority of protein consumption towards the evening meal. Recent studies have also demonstrated that a more optimal provision and timing of protein intake after exercise can significantly improve power output, muscle strength, endurance and mental alertness in subsequent bouts of exercise (Burke & Deakin, 2010; Rodriguez et al., 2009). However, during FEX individual protein intake was highly variable and did not follow any specific feeding pattern. In the context of military training, these outcomes are important considerations in temporal adaptation from the cumulative effect of arduous daily physical training and for maximal recovery between these training sessions (Rodriguez et al., 2009).

There is a vast array of evidence that suggests different distributions and absolute amounts of protein spread across the day and around physical activity could have different impacts on desired training outcome. Areta et al. (2013) demonstrated that an intake of 80 g of protein split into 20 g every 3 h resulted in a greater rate of MPS than either 8 x 10 g of protein every 1.5 h (similar to a grazing diet) or 2 x 40 g every 6 h (similar to the pattern of 'three square meals') during a 12 h recovery period from exercise. However, the studies by Areta et al. (2013) and Mamerow et al. (2014) were conducted in recreationally-active civilians who typically undertake one exercise bout per day. For this reason, these results may be more applicable only for the CAMP and MIX environments measured in the current study. In contrast, when military personnel are required to undertake multiple exercise bouts of varying intensities and durations, and at irregular times over a period of 24 hours, nutritional strategies should alter. In trained cyclists undergoing approximately 7 hours of intense training per day (similar to that of OCs), an increased protein intake attenuated a post-training decrement in time trial performance and more effectively restored performance during a subsequent week of recovery in comparison to a control group (Witard et al., 2011). Therefore, consuming a greater intake (> 20g) of protein per meal, and at multiple periods throughout the day, may have benefits to OCs and be vital for prolonged work and recovery in field settings. In the present study, protein intake was considerably lower in the evening, post-dinner (S4), for all three conditions, despite EE remaining high during these periods. Protein intake immediately prior to sleep has been shown to be effectively digested and absorbed, increasing amino acid availability (Trommelen & van Loon, 2016) and augmenting MPS rates overnight (Gillen et al., 2017) by up to 22% compared to a non-caloric placebo treatment (Trommelen & van Loon, 2016). Res et al. (2012) showed that intake of 40 g of protein prior to sleep stimulates overnight MPS rates to a greater extent than 20 or 30 g. In military populations, pre-sleep / late-evening may represent an opportunity to increase overall protein provision and improve potential for skeletal muscle adaptation to physical training.

The regular intake of protein and carbohydrate at a ratio of 1:3 to 1:4 (protein: carbohydrate) (Tarnopolsky et al., 2005) during exercise has been shown to possibly reduces Exercise-Induced Muscle Damage (EIMD) (Baty et al., 2007) and increases MPS (Tipton et al., 2004; Tipton et al., 2001). Combined intake of carbohydrate and protein stimulates muscle glycogen resynthesis to a greater extent than carbohydrate intake alone (Ivy et al., 2002), leading to increased endurance performance (Ivy et al., 2003; Saunders et al., 2004), reductions in muscle damage (Saunders et al., 2004) and greater training adaptations during acute (Bird et al., 2006a, 2006c) and prolonged exercise (Bird et al., 2006b). On FEX, although average intake of carbohydrate and protein appears optimal for these recommendations, inspecting individual intake shows a varied distribution throughout the day, and is therefore unlikely to potentiate recovery for continued work especially given that total EI was not sufficient to maintain EB.

There are of course limitations in the present study. Firstly, due to the nature of the data collection in both a field- and training-based setting, breakage and loss of activity monitoring devices meant that EE data were affected. In some respect, this data loss was also due to the wear-time cut-off of < 65%, but this cut-off is necessary to avoid including inaccurate, low daily EE and skewing overall estimation. Despite these limitations, for the aims of this specific study, the authors believe that the tri-axial accelerometer was the most practical way to accurately estimate hourly EE in field settings without undue burden to participants. It is acknowledged that food diaries can underestimate dietary intake (Hill & Davies, 2001; Tharion et al., 2004) and that it is possible that the burden of weighing food influenced participants' behaviour and eating habits (such as reduced intake in the dining hall), and therefore may not have accurately represented their usual intake. During FEX, although the use of wrapper collection likely limited the underestimation of intake, the time in which the participant ate the food items was not always recorded / were retrospectively recorded when the researcher reminded the participant when collecting the wrappers. It is therefore likely, due to sleep deprivation and long waking hours, that the time food items were eaten may have occasionally been inaccurate.

7.6 Conclusion

In conclusion, the present study is the first to document the total, and daily distribution of dietary intake in parallel with physical activity levels during three different military training settings. Total energy expenditure was greatest during military FEX, where OCs were kept active throughout the entire day, including during the night and early mornings, but this work pattern also resulted in variable patterns of dietary intake both within- and between- participants. In a more structured setting where sleep was not disrupted, a more typical three-meal eating pattern was observed. It appears that the daily distribution of dietary intake in all three military settings (CAMP, FEX and MIX) could be adapted to optimise recovery and long-term adaptations to training. Future research should evaluate potential strategies to improve the daily distribution of energy and macronutrient intake in military settings and to explore whether such interventions could enhance recovery and adaptation during training.

The finding from studies 1 to 4, demonstrated that OCs were typically in negative EB and low Energy Availability (EA) during arduous field exercises, whilst intake and distribution of macronutrients is suboptimal. These results informed the design of Study 5, which aimed to investigate whether a protein-rich supplement could reduce negative EB during an arduous field exercise as well as improve recovery of EIMD post-exercise.

7.7 Military Relevance and Recommendations

Based on the data presented in this study it is recommended that where possible military personnel take the following approaches to optimally distribute nutritional intake during periods of negative energy balance to ensure adaptation and recovery from training;

- Ensure intake is spread evenly throughout the day and a protein a carbohydrate portion is consumed during each meal
- Consume nutrient rich food before during and after exercise
- During arduous exercise where time to eat may be limited, consume ‘easy to eat’ items on a regular basis (*i.e.,* Peanuts) with a higher fat intake to mitigate any possibility of negative energy balance.

Chapter 8

Study 5

The Effect of a Protein-Rich Supplement on Energy and Macronutrient Intake and Muscle Function and Soreness during a British Army Field Exercise

8.1 Abstract

Background: Long periods of sustained low-to-moderate physical activity and sleep deprivation, particularly during military training exercises and operations, can result in high daily Energy Expenditure (EE) that are difficult to match with Energy Intake (EI), resulting in negative Energy Balance (EB) and low Energy Availability (EA). This energy deficit can contribute to negative protein balance, loss of muscle mass, and physical performance decrements. Nutritional strategies such as increasing protein intake may be used as a strategy to mitigate the effects of negative EB and low EA. The aim of the present study was to investigate the effect of dietary protein supplementation on energy and macronutrient intake, and muscle function and soreness during an arduous military field exercise. **Method:** Thirty male British Army Officer Cadets (OCs) volunteered for the study and were randomly assigned to a control group (CON; no supplement; mean ± SD; age 23 ± 2 y, body mass 80.8 ± 6.6 kg, height 1.82 ± 0.07 m) or supplementation group (SUP; age 25 ± 3 y, body mass 84.4 ± 12.5 kg, height 1.85 ± 0.08 m) who received two protein-rich food bars (217 kcal, 23.3 g protein, 13.6 g carbohydrate and 8.2 g fat) per day for 8 days during a 2-day arduous field exercise (FEX) and 4 days post exercise (Recovery). All participants were issued with a tri-axial accelerometer to measure EE, and EI was measured using food diaries and collection of food wrappers in zip-lock bags throughout the 8-day period. Exercise-induced muscle damage was inferred from Isometric Mid-Thigh Pull (IMTP) force, isokinetic knee extension / flexion peak torque, and perceived muscular soreness and fatigue ratings, which were taken at baseline, after FEX and Recovery. **Results:** Participants were in negative EB during FEX irrespective of group (CON: -3717 ± 687 vs. SUP: -3638 ± 1194 $kcal \cdot d^{-1}$; $p > 0.05$). All participants were in a low EA (≤ 30 $kcal \cdot kg\ FFM^{-1} \cdot d^{-1}$) during FEX compared to Baseline and Recovery ($p < 0.05$), however there was no difference between CON and SUP in level of EA ($p < 0.05$). At baseline, there was no difference in EI (CON: 3897 ± 505 vs. SUP: 4233 ± 765 $kcal \cdot d^{-1}$; $p > 0.05$), carbohydrate intake (CON: 439 ± 65 vs. SUP: 460 ± 122 $g \cdot d^{-1}$; $p > 0.05$) or fat intake (CON: 166 ± 23 vs. 180 ± 43 $g \cdot d^{-1}$; $p > 0.05$) between CON and SUP, however, protein intake was greater in SUP (SUP: 162 ± 32 $g \cdot d^{-1}$ vs. CON: 129 ± 30 $g \cdot d^{-1}$; $p < 0.05$). Protein intake was also greater in SUP compared to CON during FEX (CON: 56 ± 22 vs. SUP: 105 ± 30 $g \cdot d^{-1}$; $p < 0.001$) and Recovery (CON: 125 ± 37 vs. SUP: 161 ± 35 $g \cdot d^{-1}$; $p < 0.05$), as designed. Isometric knee extension at a velocity of at 180 °/s was greater during Recovery than during FEX only, but with no difference between groups. There was no difference in any other muscle function measures between conditions ($p > 0.05$) or group ($p > 0.05$). Muscular soreness and fatigue were increased after FEX ($p < 0.05$), irrespective of group ($p > 0.05$). **Conclusion:** Short periods of energy deficit do not appear to translate to any decrements in performance. However, despite a protein rich supplement reducing energy deficit and increasing protein intake, this did not appear to

alter muscle function indices during and after exercise. However, improving protein balance and energy surplus may be an effective strategy for longer term recovery.

8.2 Introduction

Long periods of sustained low-to-moderate physical activity and sleep deprivation, particularly during training exercises and operational tasks, can result in high daily Energy Expenditure (EE) that are difficult to match with Energy Intake (EI) (Margolis et al., 2013; Nindl et al., 2007). The resultant energy deficit can lead to negative Energy Balance (EB) and low Energy Availability (EA) (Torstveit et al., 2018; Wade & Jones, 2004). Studies 1 - 4 in this thesis have shown that during times of arduous training EI is sometimes not adequate to meet the demands of EE and distribution of energy and macronutrient intake in relation to physical activity during the day may be sub-optimal.

Typically during periods of high EE and low EI, Muscle Protein Synthesis (MPS) decreases and Muscle Protein Breakdown (MPB) increases, leading to an overall decline in net protein balance (Carbone et al., 2012). Negative EB, low EA and a decline in net protein balance can lead to a decrease in body mass through loss of Fat Mass (FM) and / or Fat Free Mass (FFM) (Hall et al., 2011; Hector et al., 2017). A loss in FFM adversely impacts health (Dallosso & James, 1984; Kurpad et al., 2007; Loucks, 2003), increases injury risk (Deutz et al., 2000), and impairs physical performance (Montain & Young, 2003).

Consuming dietary protein at levels exceeding the Recommended Dietary Allowance (RDA) during negative EB, upregulates whole-body protein turnover, improving net protein balance (Gaine et al., 2006) and minimising the loss of muscle protein (Carbone et al., 2012). Protein supplementation following two weeks of a 28 - 34% energy deficit was shown to restore MPS to rates observed during EB (Hector et al., 2017). However, the extent to which these metabolic processes are affected depends largely upon the degree in which energy is restricted (Pasiakos et al., 2010).

In Study 1 of this thesis it was demonstrated that it is difficult to achieve an adequate protein intake, particularly in situations where time / opportunity to eat is limited such as during Field Exercise (FEX). Additional supplemental foods have been demonstrated to be an effective strategy for mitigation of body mass loss (Tassone & Baker, 2017). Consuming dietary protein at levels exceeding the RDA (0.8 $g \cdot kg \cdot d^{-1}$) during negative EB, has been shown to be an effective nutritional countermeasure to attenuate MPB and protect muscle mass and function (Margolis, Crombie, et al., 2014; McLellan, 2013; Montain & Young, 2003), which is crucial in maintaining military performance (Henning et al., 2011; Longland et al., 2016) and decreasing susceptibility to injury (Pasiakos, Cao, et al., 2013; Tharion et al., 2005). Previous research during a British Army field exercise investigated the effect of a mixed nutritional supplement (~45% carbohydrate, ~15% protein and ~40% fat) on body composition changes and physical performance whilst soldiers were in a negative EB (Fortes et al., 2011). Although

EB was not directly investigated in the aforementioned study, previous research on the same course found that participants were in a negative EB of 644 kcal·d^{-1} over the 8-week course (Richmond et al., 2010). Fortes et al. (2011) showed that over an 8-week training course the control group that received no supplement lost a greater amount of body mass (5.0 ± 2.3 kg) and FFM (2.0 ± 1.5) compared to the supplement group (1.6 ± 1.5 kg and 0.7 ± 1.5 kg), alongside larger decrements in physical performance. Although the results cannot determine whether the protein within the supplement was responsible for the attenuation of adverse changes or whether the overall increase in calories blunted any negative outcomes caused by an energy deficit, it is clear that within the military field setting, increasing caloric intake is essential to mitigate the negative effects of a negative EB, potentially regardless of macronutrient source. Thus, nutritional interventions are likely to enable soldiers to better tolerate the high external workloads associated with military training and result in improved functional adaptation, reduced Musculoskeletal Injury (MSKI) and higher retention, resulting in greater overall operational effectiveness.

Prolonged exercise during military training, such as load-carriage, has been shown to reduce muscle force-producing capability following exercise which is indicative of Exercise-Induced Muscle Damage (EIMD) and can last up to 48 - 72 h after the initial exercise bout (Blacker, Fallowfield, et al., 2010). This reduction in force-producing capability reduces the ability of the muscles to absorb eccentric force which may result in further EIMD, increase muscle strain injury, or transfer forces to other body structures (e.g., ligaments, bones) causing subsequent overuse or traumatic injury (Blacker, 2017; Knapik et al., 2002). A large number of critical occupational tasks involve muscular strength (Hauschild et al., 2016) and therefore changes in force and power post-exercise is of primary importance to be able to perform these occupational tasks at any time. Measurements of functional impairments are most accurately assessed using voluntary and electrically stimulated contractions (Warren et al., 1999). Tests involving maximal isometric and isokinetic contractions are preferred for their reduced injury risk (Brady et al., 2018; Brown, 2000), simple administration and high test-retest reliability compared to dynamic tests such as counter movement jump peak power (Mcguigan et al., 2010; Stone et al., 2004).

Nutritional interventions following load carriage have been shown to enhance recovery of the force-producing capability of skeletal muscle following load carriage (Blacker, Fallowfield, et al., 2010) and thus reduce injury rates during military training (Flakoll et al., 2004). Protein intake is crucial for metabolic health and performance, and may be used as a strategy to preserve or increase skeletal muscle mass during times of negative EB (Longland et al., 2016), as well as mitigating MSKI risk after strenuous exercise by enhancing recovery of the force-producing capability of skeletal muscle

(Blacker, Williams, et al., 2010). Dietary protein intake after daily training sessions, particularly if participants are in a negative EB, has been shown to be beneficial for improving muscle strength and function as well as reducing muscle soreness (Pasiakos, Lieberman, et al., 2014), all of which are variables commonly used to assess EIMD (Howatson & Van Someren, 2008). Co-ingestion of carbohydrate and protein reduced EIMD during prolonged endurance exercise (Howatson & Van Someren, 2008). However, there is limited research on the use of protein supplementation during military field exercise on physical performance. Therefore, the aim of the present study is to investigate the effect of dietary protein supplementation on energy and macronutrient intake and muscle function and soreness during an arduous military field exercise.

8.3 Methods

8.3.1 *Participants*

Thirty male British Army Officer Cadets (OCs; CON: age 23 ± 2 y, body mass 80.8 ± 6.6 kg, height 1.81 ± 0.07 m; SUP: age 25 ± 3 y, body mass 84.4 ± 12.5 kg, height 1.85 ± 0.08 m) undertaking the 44-week Commissioning Course (CC) at the Royal Military Academy Sandhurst (RMAS) volunteered to participate in this study. The 30 participants were divided randomly into either a control group (CON: n = 15) or a supplementation group (SUP: n = 15). Ethical approval was granted by the Ministry of Defence Research Ethics Committee (protocol 931/MoDREC/18). Participants were provided with a verbal and written brief on the requirements of the study and were offered the opportunity to ask questions before providing informed written consent.

8.3.2 *Study Design*

The study was conducted during a mandatory arduous FEX, named Exercise Longreach, that takes place within week eight of the CC, which Study 1 demonstrated to be the most physically demanding exercise throughout the 44-week course (Figure 4.2 in Chapter 4). Exercise Longreach requires OCs to walk whilst carrying an external load in the webbing and backpack over a pre-planned route of approximately 70 km, whilst also undertaking cognitive and physically demanding tasks at pre-determined checkpoints, over a time-frame of no longer than 36 hours. The data collection schedule, as summarised in Figure 8.1, was divided into three phases, (i) Baseline (day 1 - 2), (ii) Field Exercise (FEX; day 2 - 4), and (iii) Recovery (day 4 - 8). Participants in SUP were given two commercially available protein bars (Grenade®, Solihull, UK) per day, each containing 217 kcal, 23.3 g protein, 13.6 g carbohydrate and 8.2 g fat, which they were instructed to consume between main meals for the entirety of the study, whilst eating their other food *ad libitum*. The participants received the bars every morning from the researchers whilst in camp, and in advance during FEX. Participants in CON, received no bars and were instructed to continue to follow their habitual intake.

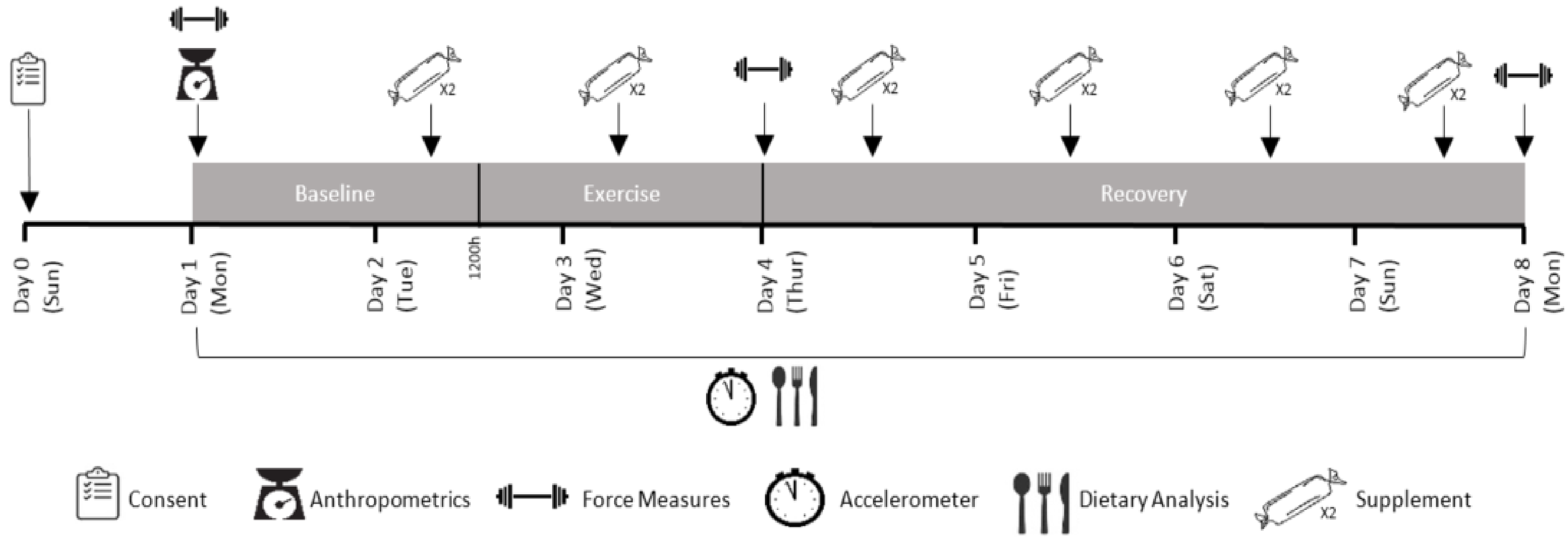

Figure 8.1: *Schematic of study design for Study 5*

8.3.3 *Anthropometry and Body Composition*

At Baseline, participants reported to the laboratory where their height was measured using a stadiometer (SECA, Birmingham, UK), body mass measured on weighing scales (SECA, Birmingham, UK) and body composition measured using Duel Energy X-ray Absorptiometry (DXA) (Lunar iDXA, GE Healthcare, UK).

8.3.4 *Energy Expenditure*

Estimated EE over the training course was estimated using a wrist-worn tri-axial accelerometer as described in Section 4.3.3 in Chapter 4. In addition, EE was also reported as average physical activity levels (PAL), which was calculate by daily EE divided by Basal Metabolic Rate (BMR), to reduce the influence of body mass on the results. Basal Metabolic Rate was calculated using the Henry equation described in Section 6.3.6 in Chapter 6.

8.3.5 *Dietary Intake*

Dietary intake was measured using food diaries and collection of all food wrappers and ration packs. On day 0, participants were provided with a brief on the food diary method and the diaries were checked by a researcher with the participant after completion on each day. During FEX, participants were provided with 24-hour ration packs, which have an energy provision of 4000 kcal (130 g protein, 651 g carbohydrate and 92 g fat) across all variations of meals and are designed to be nutritiously balanced if consumed in their entirety each day. Participants were also permitted to bring additional non-perishable foods of their choice. Researchers provided participants with a zip-lock plastic bag each day to retain discards upon which they would also record the time eaten, from all ration pack items and additional food. All discards were collected daily by the researchers and weighed. Participants' food intake was analysed using dietary analysis software (Nutritics, Nutritics LTD, Ireland) to estimate energy and macronutrient intake. A 60% compliance level was set, where if a participant did not complete a food diary for 70% of the whole data collection period, they were excluded from any dietary analysis.

8.3.6 *Energy Balance*

Daily EB was calculated using the Equation 5.1 in Chapter 5. Daily EB was average over the entirety of the exercise (1.5 days; 1200h on day 2 to 2359h on day 3), and over the recovery period (4 days).

8.3.7 *Energy Availability*

Energy availability was estimated through the Equation 6.1 in Chapter 6. Daily EA was averaged over the entirety of the exercise (1.5 days), and over the recovery period (4 days), where optimal EA for healthy physiological function is ≥ 45 kcal·kg $FFM^{-1}·d^{-1}$, reduced EA is between 30 - 45 kcal·kg $FFM^{-1}·d^{-1}$ and low EA is ≤ 30 kcal·kg $FFM^{-1}·d^{-1}$.

8.3.8 *Muscle Function, Soreness and Fatigue*

Muscle force measures were used to quantify the change in muscle function from Baseline to FEX and Recovery (Day 1, 4 and 8) using an Isometric Mid-Thigh Pull (IMTP) and knee extensions on an isokinetic dynamometer.

The IMTP was conducted with participants standing on a force plate (AWM Ltd, UK) positioned in a custom testing rig (Absolute Performance Ltd, Cardiff UK) with a bar positioned at mid-thigh height. Participants body mass was initially measured and the force plates then zeroed before participants assumed a position which replicated the second position of the clean pull, maintaining an upright trunk position (Haff et al., 1997), with a knee angle between 130^o and 140^o (Haff et al., 2008) and a hip angle of between 124^o and 175^o (Beckham et al., 2013). Participants were instructed to pull 'as hard and fast' as possible against a static bar, while maintaining the same body position and maintaining the effort for 5 seconds. Force-time characteristics during the contractions were measured using the force plates (Brady et al., 2018).

Isokinetic knee extension / flexion peak torque was measured on a randomly-allocated subset of participants (n = 16; 8 participants were measured in each group) due to time restraints in camp. Isolated right leg quadriceps and hamstring strength were investigated using an isokinetic dynamometer (Biodex System 4 Pro, IPRS Mediquipe, UK). Participants were instructed to sit in the chair with their back fully against the back rest; the chair height and seat back were adjusted to ensure the medial condyle of the right knee was in line with the middle of the dynamometer arm. Correct positioning was confirmed by moving the leg across the range of movement and observing alignment. The participant was strapped in tightly across the chest, waist and the upper right leg to restrict excess body movement that may affect the strength of the isolated leg. Toward and away limits were set, ensuring again that the participant did not experience any discomfort throughout the range of motion, and the leg was weighed in the fully-extended position. The test was preceded by two trial repetitions, where the participants were instructed to refrain from maximal effort. Following completion of the trial reps, the leg was held in the "towards" position by the researcher, after which the participants were prompted to begin the test. The test consisted of the following components: 6 repetitions at 60°/s followed by 15 repetitions at 180°/s. The tests were separated by a 2-minute rest period and standardised encouragement was given throughout the test.

On each occasion before muscle function was measured, subjective measures of muscle soreness and fatigue were recorded using a Perceived Muscle Soreness Scale whilst participants assumed a squat position (Impellizzeri & Maffiulett, 2007), and the Rating-of-Fatigue Scale (Micklewright et al., 2017). Perceived muscle soreness was estimated using a continuous range, where participants rated the soreness of their lower body on a scale of 1 to 10 (1 representing "no soreness-no impact on my training," 3 representing "soreness - minimal impact on training," 5 representing "sore - noticeable during training," 7 representing "very sore - uncomfortable to train", and 10 representing "so sore - I am unable to train"). The rating of fatigue scale was estimated using a ten-point scale where participants would rate how physically tired they felt (0 representing "Not tired at all", and 10 representing "Very tired").

8.3.9 *Data Analysis*

Results are reported as mean ± standard deviation unless otherwise stated. Sample sizes were determined *a priori* using GPower (Faul et al., 2007) in order to achieve adequate statistical power to assess differences in muscle function between CON and SUP, with $\alpha = 0.05$ and $1-\beta = 0.80$. With 12 participants required per group from the power calculation, and taking into account potential attrition (~25%) based on similar previous studies, the authors intended to recruit n = 30 with 15 participants in each group. Data for EE and EI was averaged over 1.5 days (0000 h day 1 - 1200 day 2) for Baseline, 1.5 days for FEX (1200 h day 2 - 2359 h day 3), and 4 days for Recovery (0000 h day 4 - 0000 h day 8; Figure 8.2). Data were assessed for normality, and any non-normally distributed data (EI, protein intake and carbohydrate intake) were corrected with the use of natural logarithmic transformation to ensure that kurtosis and skewness were within the normal bounds. All data were analysed with a repeated measures two-way Analysis of Variance (ANOVA), with reported effect size (partial eta squared [η^2_p]), between groups (CON and SUP) and time (Baseline, FEX and Recovery) as the main variables. Homogeneity of variances was confirmed using Levene's test. Significant interactions were analysed with Bonferroni *post hoc* tests to determine the location of the pairwise differences. Planned t-tests with reported effect sizes (Cohens D) were used to compare differences between groups. Interpretation of Cohen's d is as follows: ≤ 0.2 trivial effect , 0.21 - 0.50 small effect, 0.51 - 0.80 moderate effect and ≥ 0.8 a large effect (Cohen, 1988). Statistical analysis was conducted using

Statistical Package for the Social Sciences (SPSS; IBM SPSS version 23 for Windows, IBM Corporation, IL) and statistical significance was set *a priori* at $p < 0.05$.

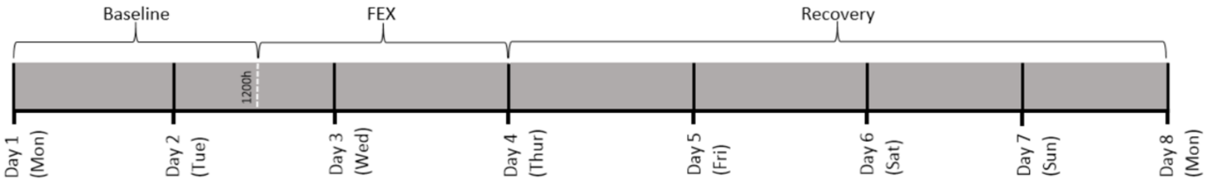

Figure 8.2: *Time period of Baseline, Field exercise (FEX) and Recovery*

8.4 Results

Due to lack of compliance of the measurements of EI, EE and DXA, one participant in CON was excluded from the whole analysis. Table 8.1 shows the number of participants included in each separate analysis. Four participants were excluded from the EE analysis due to their accelerometers not recording any data, and four participants were excluded from EI and macronutrient analysis, two due to having less than 60% compliance in food diaries and two due to missing data at Baseline. Three of the participants excluded from the EE analysis were separate to the participants that were excluded from EI analysis, therefore, due to EB calculations needing both variables, only seven participants were included in EB analysis in CON. Due to being admitted to the medical centre after FEX two participants were not included in the IMTP or muscle and fatigue measures, one of which was not included in the Biodex measurements. The distribution of EI and macronutrients were also excluded in the analysis, as the participant only recorded food diaries for 3 whole days.

Table 8.1: *Number of participants in the Control (CON) and Supplementation (SUP) group included in each analysis*

	CON	**SUP**
Energy Expenditure	10	15
Dietary Intake	10	15
Energy Balance / Availability	7	15
Isometric Mid-Thigh Pull	12	15
Isometric Knee Extension / Flexion	6	8
Soreness / Fatigue Ratings	12	15

8.4.1 *Participant Characteristics*

There were no differences in body mass ($t (24) = -1.14$, $p = 0.267$, $d = -0.440$), height ($t (24) = -0.676$, $p = 0.505$, $d = -0.262$), FM ($t (24) = 0.008$, $p = 0.993$, $d = 0.003$) or FFM ($t (24) = -1.487$, $p = 0.149$, $d = -0.576$) between CON and SUP (Table 8.2).

Table 8.2: *Participant characteristics, Fat Mass (FM) and Fat Free Mass (FFM) of participants (mean ± SD) in the Control (CON) and Supplementation (SUP) groups*

	CON	**SUP**
Participants (n)	14	15
Age (y)	23 ± 2	25 ± 3
Height (m)	1.81 ± 0.07	1.85 ± 0.08
Body mass (kg)	80.8 ± 6.6	84.4 ± 12.5
FM (kg)	14.1 ± 2.8	14.0 ± 4.8
FFM (kg)	63.0 ± 5.5	66.8 ± 8.5

8.4.2 *Energy Expenditure*

Figure 8.3 shows EE, EI and EB during baseline, FEX and recovery in the CON and SUP conditions. For EE, there was a main effect of time irrespective of group ($F_{(2,46)} = 314.85$, $p < 0.001$, $\eta^2_p = 0.932$), where EE during FEX was greater than both Baseline (mean difference [95% CIs]: 1524 [1324 - 1724], $p < 0.001$, $d = -3.919$) and Recovery (1830 [1602 - 2059], $p < 0.001$, $d = 4.117$), and Baseline was greater than Recovery (306 [167 - 445], $p < 0.001$, $d = 1.133$; Figure 8.3A). However, no main effect of group ($F_{(1,46)} = 3.65$, $p = 0.068$, $\eta^2_p = 0.137$) and no interaction effect was seen ($F_{(2,46)} = 0.01$, $p = 0.932$, $\eta^2_p = 0.000$). Planned t-tests demonstrated that there was no difference in EE between SUP and CON during Baseline ($t(23) = -1.95$, $p = 0.063$, $d = 0.043$) and FEX ($t(24) = -1.40$, $p = 0.175$, $d = 0.244$), however EE was greater in SUP compared to CON during Recovery ($t(24) = -2.34$, $p = 0.028$, $d = -0.929$), as shown in Figure 8.3.

For PAL there was a main effect of time ($F_{(2,46)} = 359.83$, $p < 0.001$, $\eta^2_p = 0.940$) irrespective of group, where FEX was greater than Baseline (mean difference [95% CIs]: 0.60 [0.50 - 0.70], $p < 0.001$, $d = 3.170$) and Recovery (0.37 [0.29 - 0.45], $p < 0.001$, $d = 2.445$), and Baseline was greater than Recovery (0.97 [0.88 - 1.11], $p < 0.001$, $d = 5.127$).

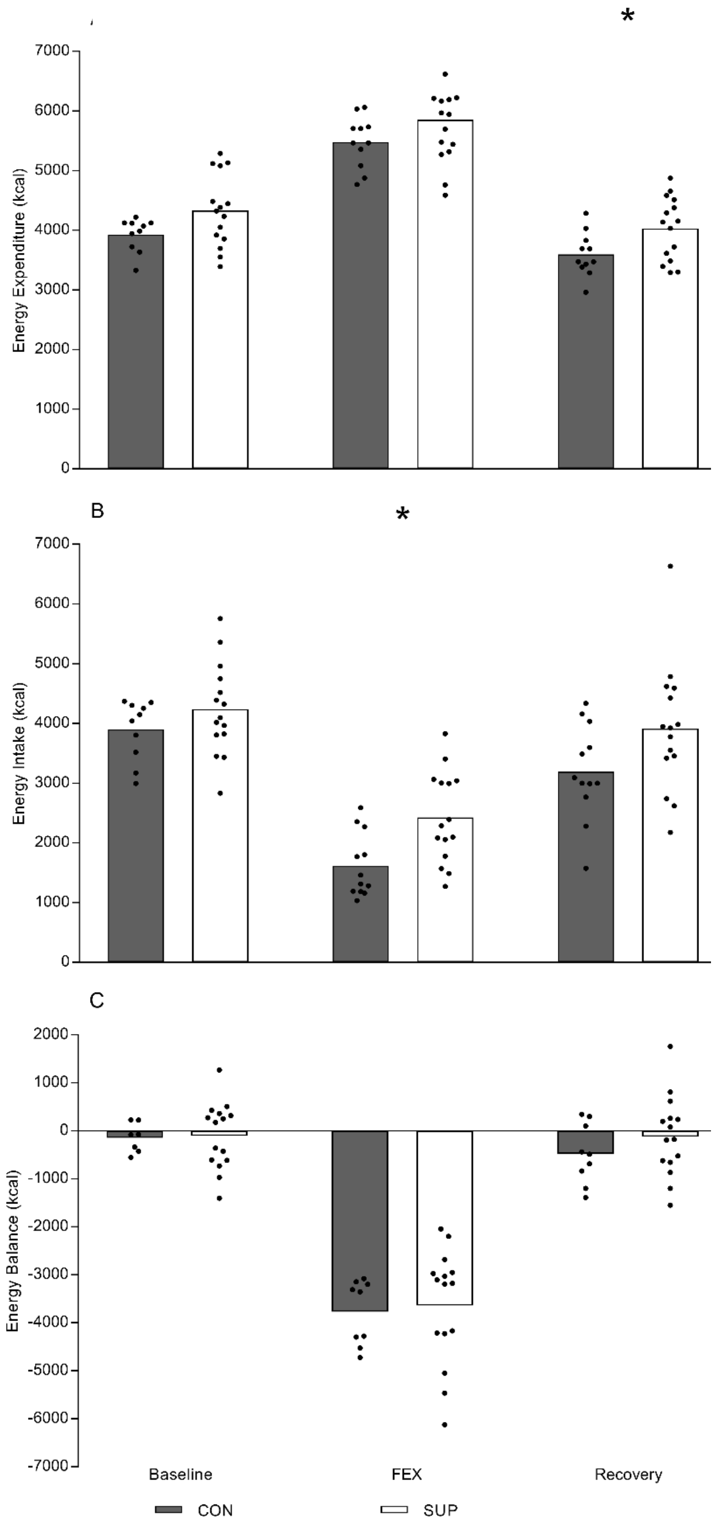

Figure 8.3: *Energy Expenditure (EE; A), Energy Intake (EI; B) and Energy Balance (EB; C) during Baseline, Field Exercise (FEX) and Recovery in the Control (CON; ■) and Supplementation (SUP; □) groups. * represents a statistical significance between groups, p < 0.05*

However, there was no main effect of group ($F_{(1,23)}$ = 4.02, p = 0.057, η^2_p = 0.149) or interaction effect ($F_{(2,46)}$ = 0.36, p = 0.700, η^2_p = 0.015). Planned t-tests showed that there was no difference in PAL between groups during Baseline (CON: 2.4 ± 0.2 vs. SUP: 2.5 ± 0.2; t (23) = -1.84, p = 0.079, d = -0.75) or FEX (CON: 3.0 ± 0.2 vs. SUP: 3.1 ± 0.2; t (24) = -1.00, p = 0.327, d = -1.179). However, PAL was greater in SUP compared to CON during Recovery (CON: 2.0 ± 0.2 vs. SUP: 2.1 ± 0.2; t (24) = -2.150, p = 0.042, d = -1.659).

8.4.3 *Energy Intake*

For EI, there was a main effect of time ($F_{(2,46)}$ = 49.33, p < 0.001, η^2_p = 0.682) irrespective of group, where EI during Baseline was greater than both FEX (mean difference [95% CIs]: 2009 [1516 - 2502], p < 0.001, d = 2.099) and Recovery (520 [70 - 970], p = 0.020, d = 0.595), and EI during Recovery was greater than FEX (1489 [837 - 2142], p < 0.001, d = -1.175). Energy intake was greater in SUP compared to CON ($F_{(1,23)}$ = 10.40, p = 0.004, η^2_p = 0.311), irrespective of time, however there was no interaction effect ($F_{(2,46)}$ = 0.903, p = 0.412, η^2_p = 0.038). Planned t-tests demonstrated that there were no differences between CON and SUP at Baseline (t (23) = -1.22, p = 0.235, d = -0.497), however EI was greater in SUP compared to CON during FEX (t (24) = -3.11, p = 0.005, d = -1.204). There was no difference between groups during Recovery (t (24) = -1.930, p = 0.065, d = -0.748; Figure 8.3B).

8.4.4 *Energy Balance*

For EB, there was a main effect of time ($F_{(2,40)}$ = 103.82, p < 0.001, η^2_p = 0.838), irrespective of group, where EB was more negative during FEX compared to both Baseline (mean difference [95% CIs]: 3550 [2930 - 4170], p < 0.001, d = 3.177) and Recovery (3368 [2582 - 4154], p < 0.001, d = -2.377). However, there was no difference between Baseline and Recovery (p = 1.00, d = 0.185). There were no main effects of group ($F_{(1,20)}$ = 1.12, p = 0.303, η^2_p = 0.053) or interaction effects ($F_{(2,46)}$ = 0.54, p = 0.588, η^2_p = 0.026). Planned t-tests demonstrated that there was no difference in EB between groups at Baseline (t (20) = -0.16, p = 0.875, d = -0.073), FEX (t (20) = -0.16, p = 0.873, d = -0.074) or Recovery (t (20) = -1.58, p = 0.129, d = -0.725; Figure 8.3C).

8.4.5 *Energy Availability*

For EA, there was a main effect of time ($F_{(2,40)} = 107$, $p < 0.001$, $\eta^2_p = 0.843$), irrespective of group, where EA was lower in FEX compared to both Baseline (mean difference [95% CIs]: 54 [45 - 63], p < 0.001, d = 3.299) and Recovery (50 [39 - 50], p < 0.001, d = -2.515), however there was no difference between Baseline and Recovery (p = 1.00, d = 0.181). There was no difference in EA between CON and SUP ($F_{(1,20)} = 4.00$, $p = 0.059$, $\eta^2_p = 0.167$), irrespective of time, and no interaction effect ($F_{(2,40)} = 0.76$, $p = 0.473$, $\eta^2_p = 0.037$). On average, participants were in reduced EA during Baseline (CON: 30 ± 5 and SUP: 31 ± 13 kcal·kg $FFM^{-1}·d^{-1}$) where 57% and 47% of participants in CON and SUP, respectively, were classified as being in low EA, compared to during FEX where all participants in both groups were in low EA (CON: -30 ± 8 and SUP: -21 ± 16 kcal·kg $FFM^{-1}·d^{-1}$). During Recovery, on average, participants in CON were in low EA (20 ± 10 kcal·kg $FFM^{-1}·d^{-1}$; Figure 8.4), where 86% of participants were classified as being in low EA. In SUP, on average participants were in reduced EA (30 ± 13 kcal·kg $FFM^{-1}·d^{-1}$) and 47% of participants were classified as being in low EA.

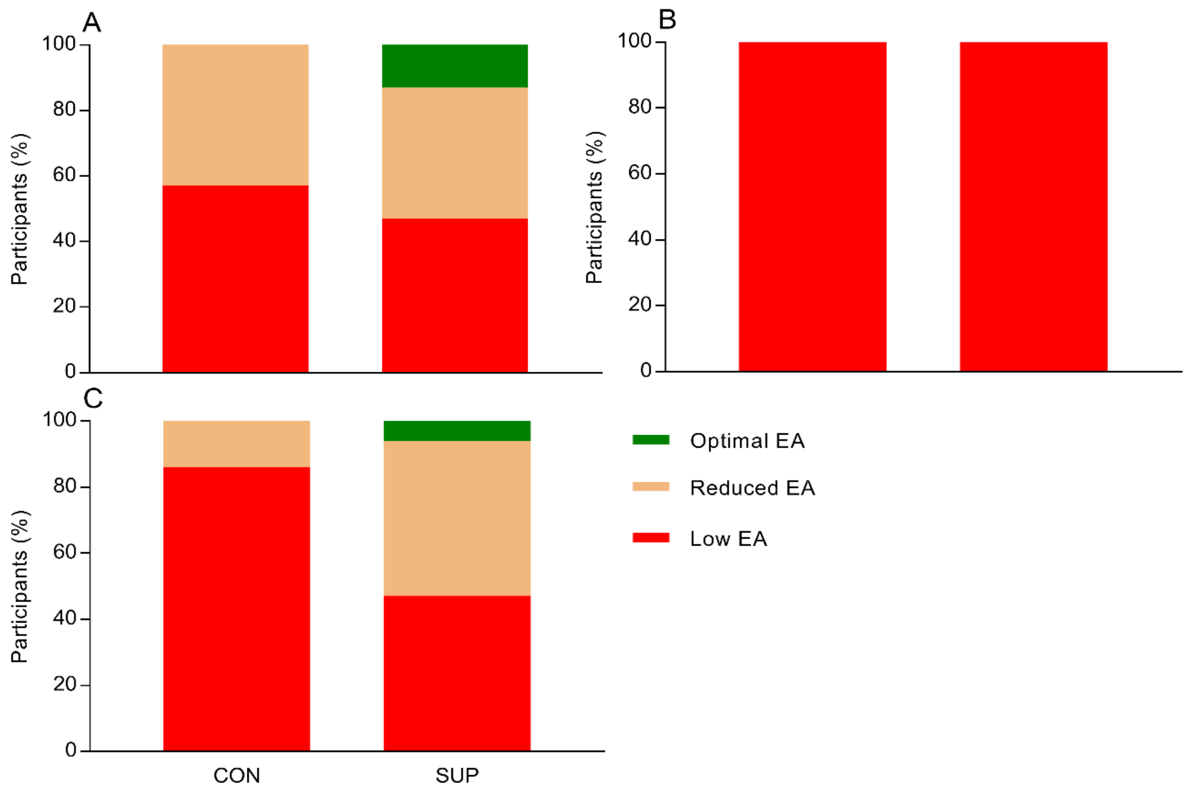

Figure 8.4: *Percentage of participants in the Control group (CON) and Supplementation group (SUP) in optimal Energy Availability (EA; ≥ 45 kcal·kg $FFM^{-1}·d^{-1}$), reduced EA (30 - 45 kcal·kg $FFM^{-1}·d^{-1}$) and low EA (≤ 30 kcal·kg $FFM^{-1}·d^{-1}$) during Baseline (A), Field Exercise (FEX; B) and Recovery (C)*

8.4.6 *Macronutrient Intake*

For total carbohydrate intake, there was a main effect of time ($F_{(2,46)} = 31.39$, $p < 0.001$, $\eta^2_p = 0.577$; Figure 8.5A), irrespective of group, where intake during Baseline was greater than FEX (mean difference [95% CIs]: 204 [137 - 272], p < 0.001, d = 1.557) and Recovery (94 [39 - 149], p < 0.001, d = 0.881), and intake during Recovery was greater than FEX (110 [34 - 185], p = 0.004, d = -0.749). There was no main effect of group ($F_{(1,23)} = 3.31$, $p = 0.082$, $\eta^2_p = 0.126$), and no interaction effect ($F_{(2,46)} = 0.69$, $p = 0.508$, $\eta^2_p = 0.029$). Planned t-tests demonstrated that there was no difference in carbohydrate intake in CON and SUP during Baseline (t (23) = -0.49, p = 0.628, d = -0.201), however intake was greater in SUP than CON during FEX (t (23) = -2.59, p = 0.016, d = -1.057; Figure 8.5A). There was no difference between groups during Recovery (t (23) = -1.14, p = 0.268, d = -0.464). Relative carbohydrate intake demonstrated similar results, where there was no difference between groups during Baseline (t (23) = -0.49, p = 0.628, d = -0.201) or Recovery (t (23) = -1.14, p = 0.268, d = -0.464), but there was a greater relative intake in SUP compared to CON during FEX (t (23) = -2.59, p = 0.016, d = -1.057; Table 8.3).

For total protein intake, there was a main effect of time, irrespective of group ($F_{(2,46)} = 44.74$, $p < 0.001$, $\eta^2_p = 0.660$; Figure 8.5B), where intake was lower during FEX compared to Baseline (mean difference [95% CIs]: 64 [42 - 85], p < 0.001, d = 0.525) and Recovery (61 [34 - 89], p < 0.001, d = -5.732), however there was no difference between Baseline and Recovery (p = 1.00, d = -1.146). Protein intake was greater in SUP compared to CON ($F_{(1,23)} = 44.50$, $p < 0.001$, $\eta^2_p = 0.605$), irrespective of time. A group-time interaction ($F_{(1.59,36.55)} = 4.53$, $p = 0.024$, $\eta^2_p = 0.165$) was also present, where protein intake was greater in SUP than CON at Baseline (p = 0.018, d = -1.040), FEX (p < 0.001, d = -1.808) and Recovery (p = 0.021, d = -1.009), but the changes between Baseline and FEX (-57%; p < 0.05) and between FEX and Recovery (125%; p < 0.05) in CON were larger than in SUP (-33% vs. 53%; Figure 8.5B). Relative protein intake demonstrated similar results whereby protein intake was greater in SUP than CON at Baseline (t (23) = -2.548, p = 0.018, d = -1.040), FEX (t (23) = -4.428, p < 0.001, d = -1.808), and Recovery (t (23) = -2.471, p = 0.021, d = -1.009; Table 8.3).

For fat intake, there was a main effect of time ($F_{(2,46)} = 32.84$, $p < 0.001$, $\eta^2_p = 0.588$; Figure 8.5C), irrespective of group, where Baseline was greater than FEX (mean difference [95% CIs]: 92 [68 - 116], p < 0.001, d = 1.992) and Recovery (34 [5 - 64], p = 0.020, d = 0.593), and intake during Recovery was greater than FEX (58 [35 - 91], p < 0.001, d = -0.897). Fat intake was greater in SUP compared to CON ($F_{(1,23)} = 7.682$, $p = 0.011$, $\eta^2_p = 0.250$), irrespective of time. However, there was no interaction effect ($F_{(2,46)} = 0.55$, $p = 0.583$, $\eta^2_p = 0.023$). Planed t-tests demonstrated that SUP consumed more fat than

CON during FEX (t (25) = -2.382, p = 0.025, d = -0.923; Figure 8.5C). However, there was no difference in fat intake between groups at Baseline (t (23) = -0.98, p = 0.336, d = -0.402) or during Recovery (t (25) = -1.365, p = 0.184, d = -0.529). Relative fat intake demonstrated similar results, where SUP had a greater relative fat intake compared to CON during FEX (t (25) = -2.382, p = 0.025, d = -0.923), however there was no difference between groups at Baseline (t (23) = -0.984, p = 0.336, d = -0.402) or during Recovery (t (25) = -1.365, p = 0.184, d = -0.529; Table 8.3).

Table 8.3: *Relative Carbohydrate (rCHO), Protein (rPRO), and Fat (rFAT) intake (mean ± SD) during Baseline, Field Exercise (FEX) and Recovery, in the Control (CON) and Supplementation (SUP) group*

	CON (n = 10)			**SUP (n = 15)**		
	Baseline	**FEX**	**Recovery**	**Baseline**	**FEX**	**Recovery**
rCHO ($g \cdot kg \cdot d^{-1}$)	7.3 ± 1.1	3.3 ± 0.7	5.4 ± 1.6	7.7 ± 2.0	4.7 ± 1.6*	6.3 ± 2.4
rPRO ($g \cdot kg \cdot d^{-1}$)	2.2 ± 0.5	0.9 ± 0.4	2.1 ± 0.6	2.7 ± 0.5*	1.8 ± 0.5*	2.7 ± 0.6*
rFAT ($g \cdot kg \cdot d^{-1}$)	2.8 ± 0.4	1.0 ± 0.5	2.1 ± 0.6	3.0 ± 0.7	1.6 ± 0.6*	2.6 ± 1.0

* significant difference from CON, p < 0.05.

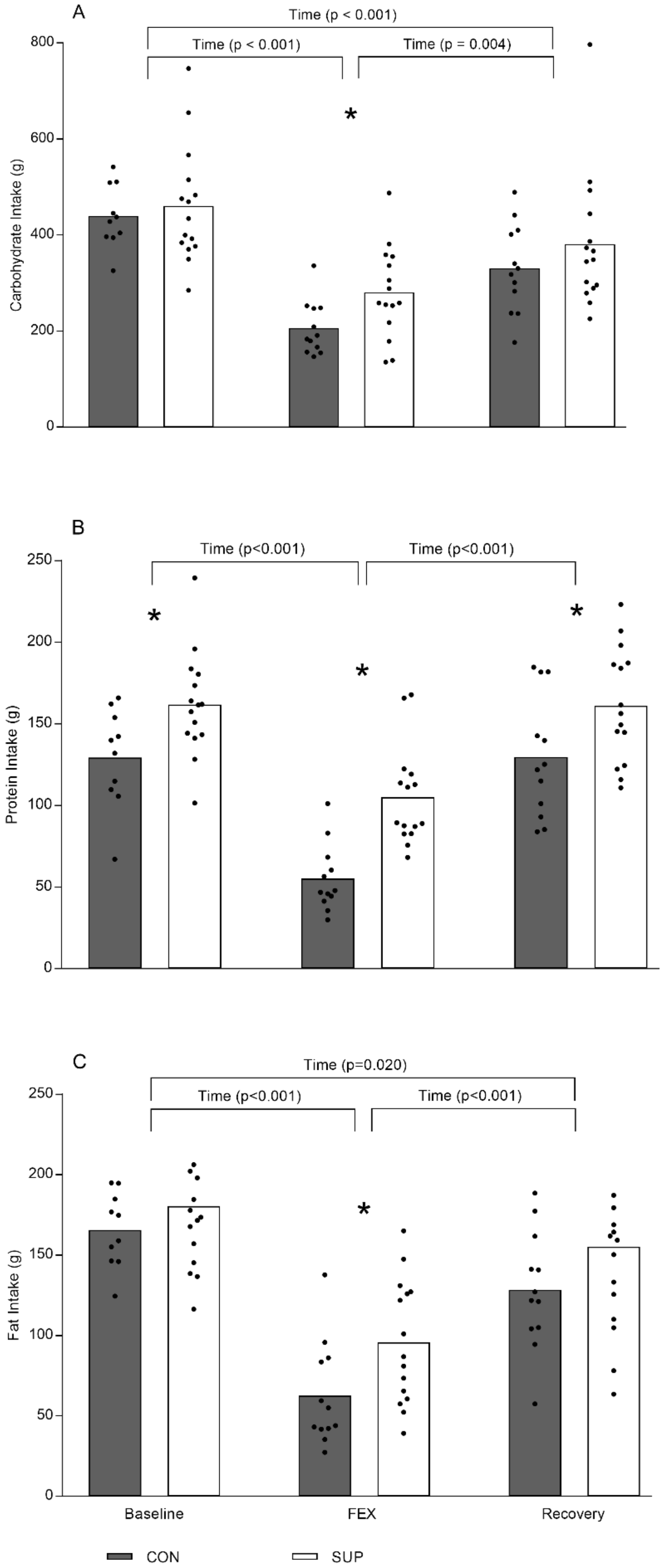

Figure 8.5: *Average and individual Carbohydrate (A), Protein (B) and Fat (C) intake during Baseline, Field Exercise (FEX) and Recovery in the Control (CON; ■) and Supplementation (SUP; □) group. * represents a statistical significance between groups, p < 0.05*

8.4.7 *Markers of Muscle Damage*

8.4.7.1 Isometric Mid-Thigh Pull

For IMTP there was no time ($F_{(2,50)} = 0.09$, $p = 0.911$, $\eta^2_p = 0.004$), group ($F_{(1,25)} = 1.59$, $p = 0.220$, $\eta^2_p = 0.060$) or interaction effect ($F_{(2,50)} = 1.72$, $p = 0.190$, $\eta^2_p = 0.064$).

Table 8.4: *Force during isometric Mid-Thigh Pull (IMTP), Biodex Extension (Ext) and Flexion (Flex) at 180 °/s and 60 °/s and subjective ratings of muscle soreness and muscle fatigue for the Control (CON) and Supplement (SUP) group during Baseline, Field Exercise (FEX) and Recovery*

	CON			**SUP**		
	Baseline	**FEX**	**Recovery**	**Baseline**	**FEX**	**Recovery**
IMTP (kg)	174 ± 18	180 ± 16	176 ± 20	193 ± 35	187 ± 34	189 ± 36
Biodex (°/s)						
Ext 60	221 ± 29	202 ± 15	217 ± 23	206 ± 45	209 ± 51	210 ± 49
Ext 180†	144 ± 24	141 ± 22	154 ± 22	150 ± 27	149 ± 28	154 ± 26
Flex 60	114 ± 10	111 ± 10	113 ± 9	106 ± 23	109 ± 30	110 ± 33
Flex 180	79 ± 10	79 ± 10	81 ± 9	85 ± 16	88 ± 15	87 ± 20
Muscle Soreness*†	2 ± 2	6 ± 1	2 ± 2	2 ± 2	6 ± 2	2 ± 2
Muscle Fatigue*†**	5 ± 2	6 ± 2	3 ± 2	4 ± 3	6 ± 2	3 ± 2

* significant difference from between Baseline and FEX, ** significant difference between Baseline and Recovery, † Significant difference between FEX and Recovery, $p < 0.05$.

8.4.7.2 Isometric Knee Extension / Flexion

For isometric knee extension at a velocity of at 60 °/s, there was no main effect of time ($F_{(2,24)} = 1.65$, $p = 0.214$ $\eta^2_p = 0.121$), group ($F_{(1,12)} = 0.06$, $p = 0.811$, $\eta^2_p = 0.005$), or interaction effect ($F_{(2,24)} = 2.24$, $p = 0.128$, $\eta^2_p = 0.157$). For isometric knee extension at a velocity of at 180 °/s there was a main effect of time ($F_{(2,24)} = 7.59$, $p = 0.003$, $\eta^2_p = 0.387$), irrespective of group, where muscular force was greater during Recovery than during FEX (mean difference [95% CIs]: 8 [2 - 15], $p = 0.012$, $d = -0.933$), however there was no difference between Baseline and FEX ($p = 1.00$, $d = 0.206$) nor Baseline and Recovery ($p = 0.105$, $d = -0.629$). There was no main effect of group ($F_{(1,12)} = 0.12$, $p = 0.740$, $\eta^2_p = 0.010$) or interaction effect ($F_{(2,24)} = 1.33$, $p = 0.284$, $\eta^2_p = 0.100$).

For isometric knee flexion at a velocity of at 60 °/s, there was no main effect of time ($F_{(2,24)} = 0.20$, $p = 0.820$, $\eta^2_p = 0.016$), group ($F_{(1,12)} = 0.127$, $p = 0.728$, $\eta^2_p = 0.010$) or interaction effect ($F_{(2,24)} = 0.68$, $p = 0.516$, $\eta^2_p = 0.054$). For isometric knee flexion at a velocity of at 180 °/s, there was also no main effect of time ($F_{(2,24)} = 0.40$, $p = 0.678$, $\eta^2_p = 0.032$), group ($F_{(1,12)} = 0.78$, $p = 0.394$, $\eta^2_p = 0.061$) or interaction effect ($F_{(2,24)} = 0.254$, $p = 0.778$, $\eta^2_p = 0.021$).

For muscle soreness, there was a main effect of time ($F_{(2,50)} = 67.28$, $p < 0.001$, $\eta^2_p = 0.729$), irrespective of group, where FEX had a greater rating of muscle soreness compared to Baseline (mean difference [95% CIs]: 4 [3 - 5], $p < 0.001$, $d = -2.182$) and Recovery (4 [3 - 5], $p < 0.001$, $d = 1.963$), however there was no difference between Baseline and Recovery ($p = 1.00$, $d = -0.017$). There was no main effect of group ($F_{(1,25)} = 1.25$, $p = 0.275$, $\eta^2_p = 0.048$) or interaction effect ($F_{(2,50)} = 0.22$, $p = 0.801$, $\eta^2_p = 0.009$). For muscle fatigue, there was a main effect of time ($F_{(2,50)} = 20.71$, $p < 0.001$, $\eta^2_p = 0.453$), irrespective of group, where FEX had a greater rating of muscle fatigue compared to Baseline (2 [0 - 3], $p = 0.006$, $d = -0.664$) and Recovery (3 [2 - 5], $p < 0.001$, $d = 1.240$), and Baseline had a greater rating compared to Recovery (1 [0 - 3], $p = 0.010$, $d = 0.624$). There was no main effect of group ($F_{(1,25)} = 0.018$, $p = 0.895$, $\eta^2_p = 0.001$) or interaction effect ($F_{(2,50)} = 0.52$, $p = 0.596$, $\eta^2_p = 0.020$).

8.5 Discussion

The present study has shown that consuming a protein-rich supplement in addition to an *ad libitum* diet during, and after, an arduous military field exercise reduced the energy deficit, and increased dietary protein intake to meet or exceed the recommended athletic guidelines. However, this change in dietary provision did not mitigate the increase in perceived fatigue and muscle soreness which resulted from the FEX. Field exercise also appeared to not reduce muscle function measured using the IMTP and isokinetic dynamometer, and therefore no discernible effect of supplementation could be detected on these performance measures.

In Study 1 of this thesis, it was reported that military personnel were in a large energy deficit (-2190 ± 436 $kcal \cdot d^{-1}$) during arduous field exercises. These energy deficits are largely driven by sustained periods of low-to-moderate physical activity that result in high daily EE that are difficult to match with EI, as time availability to eat or prepare meals are often limited (Margolis et al., 2013; Nindl et al., 2007). By consuming two protein-rich supplement bars per day over the field exercise, EI increased by approximately 434 $kcal \cdot d^{-1}$, decreasing the overall negative EB (CON: -3717 ± 687 vs. SUP: -3637 ± 1194 $kcal \cdot d^{-1}$). However, during FEX, EE was shown to be 34 and 43% greater, for CON and SUP respectively, than supplied ration packs (4000 kcal), suggesting that current provision may not be adequate for the demands of arduous field exercises.

Attempts to increase dietary intake to adequately meet EE demands can be unsuccessful if time and tactical restraints imposed on military personnel during FEX compromise the soldiers ability to consume additional food (Margolis et al., 2016). Therefore, interventions to increase dietary intake following periods of negative EB may be more effective. Berryman et al. (2017) found that after an energy deficit of -4203 ± 1686 kcal·d^{-1} for seven days, a positive EB of 977 ± 435 kcal·d^{-1} for 27 days fully restored FFM loss. In the present study during Recovery, SUP was in a 3% energy deficit compared to 19% in CON. The results suggest that implementing dietary strategies in the recovery period after periods of negative EB may be an effective way to increase EI and potentially offset any negative consequences of an energy deficit.

Similar to Study 3 (Chapter 6), EA in the present study was demonstrated to be reduced during Baseline and Recovery and low (≤ 30 kcal·kg FFM^{-1}·d^{-1}) during FEX, which is thought to disrupt and / or impair physiological, metabolic, and functional characteristics, including metabolic rate (Loucks & Thuma, 2003), immune function (Hagmar et al., 2008), protein synthesis (Areta et al., 2013), and increased injury risk (Thein-Nissenbaum et al., 2011). Gomez-Merino et al. (2002) demonstrated a decreased endocrine function during a large energy deficit (-1800 kcal·d^{-1}) in French Officer Cadets undertaking a 5-day combat course. It is therefore possible that the acute period in which low and reduced EA was experienced over the data collection period, may have elicited physiological impairment. However, despite the recent abundance in studies investigating EA in athletes, it is still unknown as to what magnitude or time course an energy deficit needs to be elicited before physiological consequences are experienced, particularly in military personnel.

Although there was no difference in EA between CON and SUP, it is thought that an increased protein intake during periods of low EA (Areta et al., 2014; Mamerow et al., 2014) may help attenuate a negative net protein balance and restore the physiological / homeostatic function. In the present study, relative protein intake in SUP was generally high and were within the athletic guidelines for protein intake (1.2 - 2.0 g·kg·d^{-1}) (Pasiakos, Montain, et al., 2013; Rodriguez et al., 2009) during Baseline, FEX and Recovery despite being in a low / reduced EA. Similar results were demonstrated in Melin et al. (2016) where a high protein intake was observed among the female endurance athletes (1.9 g·kg·d^{-1}) when in reduced EA. However, the results also may suggest that although a high protein intake may protect against FFM loss during energy deficiency, consuming a higher proportion of fat, which is more energy dense, may have better protected against low EA than consuming more protein.

In the present study, there was a twofold difference in total protein intake between CON and SUP during FEX, indicating that the supplementation was successful in eliciting a comparative increase in protein intake and suggesting that the control group was likely representative of typical nutritional composition in this setting. During times of energy restriction, protein intake of 2 - 3 g·kg·d^{-1} (Helms et al., 2014) may be needed to support adaptation to training, and counteract the negative effects of an energy deficit (Areta et al., 2014; Hector et al., 2017; Pasiakos, Cao, et al., 2013). Protein intake in the current study was below the recommended guidelines in CON (0.9 $g \cdot kg \cdot d^{-1}$) but adequate for SUP (1.8 $g \cdot kg \cdot d^{-1}$) (Pasiakos, Montain, et al., 2013; Phillips et al., 2007; Phillips & Van Loon, 2011; Rodriguez et al., 2009). A higher protein intake (> 0.8 $g \cdot kg \cdot d^{-1}$) has been shown to help attenuate muscle degradation and promote muscle anabolism during energy deficits (Areta et al., 2014; Carbone et al., 2013), as well as increase strength and power, and aid muscle recovery (Phillips & Van Loon, 2011; Rodriguez, 2013; Rodriguez et al., 2009). Interestingly, a higher protein intake of 3.0 $g \cdot kg \cdot d^{-1}$ has been reported to attenuate the decline in exercise performance during an intensified training period compared to 1.5 $g \cdot kg \cdot d^{-1}$ (Witard et al., 2011). This suggests that protein requirements during intensified training may be higher (Williamson et al., 2018), and therefore an even greater protein intake in SUP was needed to result in any performance benefits.

In the present study, although it wasn't expected that a short exercise would have elicited any reduction in FFM, it was still thought that performance decrements would have been present. It was expected that EIMD would occur during the field exercise owing to the prolonged, unaccustomed exercise involving long periods of downhill walking whilst carrying heavy loads, and would have resulted in subsequent performance decrements (Howatson & Van Someren, 2008). The lack of differences between Baseline and FEX is surprising, as previous work has demonstrated that reductions in torque are present after a 12.1 km road march carrying 27 kg load (Clarke et al., 1955), however It may be likely that OCs in the present study undertook physical activity prior to Baseline and therefore attended the study in an already fatigued state. Similar results have been demonstrated by Knapik et al. (1991), who observed no change in muscle function (vertical jump power) in military personnel following a maximal effort 20 km road march carrying 46 kg, as well by Ainslie et al. (2003) who showed no decrease in vertical jump performance for recreational walkers following a self-paced 21 km hill walk carrying 9.5 kg. In the present study, subjective measures of muscle soreness and fatigue were observed, whereby FEX resulted in a significant increase in perceived muscle soreness and fatigue; factors which are linked to EIMD (Howatson & Van Someren, 2008). Similarly, Margolis, Murphy, et al. (2014) observed similar increases in muscle soreness during a 3-day ski march, however, unlike the present study, the authors observed performance decrements (in vertical jump height). Some differences between Margolis, Murphy, et al. (2014) and the current study may explain differences in the findings, as the field exercises were different durations and conducted in different environments (temperate conditions vs. temperatures of -15^{o}) and using different activity modes (loaded marching vs. ski marching). As it has been demonstrated that skiing movements are considerably different to walking (Millet & Lepers, 2004), it is not surprising that there might be differences in performance decrements. Additionally, vertical jump height may be more representative of lower limb muscle fatigue compared to IMTP that may be more representative of relative strength (Thomas et al., 2015).

Surprisingly, in the present study, there was also no change in the measurements of muscle function between groups. Blacker, Williams, et al. (2010) investigated the effects of a protein supplement (consumed immediately before and after exercise, as well as in the evening after the testing session) on prolonged walking with load carriage and reported no difference in knee extension between a control (placebo) or supplementation (36 g of protein per dose) group at any time point. Similar results were seen in Mettler et al. (2010), who demonstrated that one week of negative EB showed no performance difference in maximal voluntary contraction force, One-Repetition Maximum (1RM), jump peak force and Wingate tests, between resistance trained men consuming a low (1 $g{\cdot}kg{\cdot}d^{-1}$) or high (2.3 $g{\cdot}kg{\cdot}d^{-1}$) protein diet during a 60% energy deficit, despite a difference in the loss of FFM between groups. In contrast, Flakoll et al. (2004) reported that in US Marine basic training, the ingestion of protein post-exercise reduced muscle soreness by 7% after a 6-mile march, and 17% on the day post compared to a control or carbohydrate group. Similar increases have been shown after ultra-marathons, and have shown to be related to markers of inflammation and EIMD (Ramos-Campo et al., 2016). Similarly, the results in the present study demonstrated that muscle soreness and fatigue, although increased by FEX, did not differ between groups. However, baseline protein intake in the latter studies were not reported, therefore the increase in protein intake from habitual intake was unknown. In the present study, it may be likely that a higher consumption of protein may be needed to elicit any benefits to performance during, and following, arduous FEX.

Although not reported here, it was hypothesised that protein supplementation would improve whole-body protein turnover, even during short periods of negative EB. Previous studies have demonstrated that over a 48-hour severe negative EB, whole-body protein breakdown was increased while synthesis was unaltered, therefore driving net protein balance to be more negative (Karl et al., 2016). Therefore, despite the lack of performance differences, intake of protein higher than the RDA during an energy deficit, may offer metabolic advantages during energy restricted states (Longland et al., 2016).

The following limitations are acknowledged in the present study. Although the sample size was justified based on the power calculation *a priori*, the calculations did not account for the unexpected large number of participants that had to be excluded from the analysis, which limited the statistical analyses that could be used. It is also possible that due to not blinding the supplement, participants' eating behaviours and habits (*i.e.,* increased protein intake in CON) may have been affected and therefore may not have accurately represented habitual intake. For instance, in SUP it may have been likely that the supplement bars were consumed at the expense of other food items that they would have habitually eaten. This has previously been observed by Margolis et al. (2016) who demonstrated that participants in the protein group consumed 500 kcal·d^{-1} less from their rations compared to the control group. Although participants were asked to consume the supplement between core meals, only 40% of participants adhered to reporting the time that food was consumed whilst on FEX. Therefore, the distribution of intake was unknown.

It is also possible that the performance measures used in the current study did not accurately represent the performance decrements that may have otherwise been seen. The exercise which the OCs undertook in the present study is a multi-faceted training exercise, which was hypothesised to elicit a decrease in dynamic strength and performance due to increased fatigue. It is therefore likely that other measures, such as vertical jump performance, that measures the active eccentric, isometric and concentric muscle contraction (Thomas et al., 2015), may have been more appropriate to use, compared to IMTP, which involves an active isometric muscle contraction only, and therefore transfers to relative strength (Thomas et al., 2015). Despite this limitation, IMTP has been demonstrated to be a relatively simple technique, with quick administration and reduced injury risk (Brady et al., 2018), and has shown to correlate well with performance on dynamic tests such as counter movement jump peak power (Mcguigan et al., 2010; Stone et al., 2004), therefore was considered the most appropriate measurement to use in the current study. Additionally, due to the study design, it is likely that some participants would have had over 12 hours between finishing the exercise and performing the muscle function measures. Therefore, it is likely that performance measures may not have happened close enough to the fatiguing point (or at enough time points) to pick up possible effects.

Lastly, even with the increase in protein, it is likely that due to the lack of body mass and FFM loss, the exercise may not have been an appropriate length to elicit any performance decrements, and therefore the total energy deficit over the entire data collection period was below the limit of -5686 to -19,109 kcal to see any effect on performance (Murphy et al., 2018).

8.6 Conclusion

In conclusion, short periods of energy deficit from this particular field exercise appear unlikely to elicit any decrements in muscle function but did increase perceived muscle soreness and fatigue. Despite a protein rich supplement reducing energy deficit and increasing protein intake, this did not appear to alter muscle function indices during and after exercise. However, improving protein balance and energy surplus may be an effective strategy for longer term recovery. Therefore, further studies are warranted to explore whether a nutritional supplement may be beneficial during longer periods of military training.

8.7 Military Relevance and Recommendations

Based on the data presented in this study, it is recommended that military personnel take the following approaches to ensure optimal nutritional intake during and after arduous exercise for the benefit of maintain and / or improving training adaptation and recovery;

- Consume nutrient rich food throughout a field exercise period and ensure a high protein and carbohydrate intake
- Consume 'easy to eat' items whilst undertaking arduous field exercises (*e.g.* Peanuts, snack bars) with a higher fat intake to mitigate any possibility of negative energy balance
- Ensure a higher energy intake with nutrient rich food is consumed post-field exercise to accelerate restoration of energy balance and energy availability.

Chapter 9

Discussion

Discussion

9.1 Discussion of Experimental Chapters

The research presented in this thesis has adopted a general approach of nutritional intake being a modifiable factor within the military environment which can be used to influence the physiological responses to external workloads and the resultant training outcomes. In order to implement evidence-based nutritional interventions to improve training outcomes in military settings the nutritional intake and physical demands have to first be quantified. Figure 9.1, illustrates how the results from Studies 1 to 4 provided new data to improve the understanding of the physical activity levels and nutritional intake of Officer Cadets (OCs) during training. These data were used to inform an evidence-based intervention in Study 5 to explore the effect of a protein-rich supplement during an arduous field exercise on Energy Intake (EI), macronutrient intake and muscle function and soreness.

Study 1 provided a summary of OCs nutritional intake and physical activity profiles during the Commissioning Course (CC) and specifically examined these variables in three different training settings; during Camp training (CAMP), Field Exercise (FEX) and a combination of both camp and field training (MIX). The study demonstrated that the Energy Expenditure (EE) over each term (Junior: 4371 ± 851, Intermediate: 3937 ± 775, Senior: 3712 ± 732 kcal·d^{-1}) were similar, if not higher, than previously reported in military training. The average EE across the measurement periods showed that the physical demands remained consistently high throughout the CC, with various spikes due to periods of demanding field exercises. It was also demonstrated that the estimated physical demands in the Study 1, were representative of the Military Dietary Reference Values (MDRVs) (SACN, 2016) for both men and women, suggesting the MDRVs are a useful guide for energy requirements of military training. Nutritional intake of OCs at the Royal Military Academy Sandhurst (RMAS) has not previously been reported. In comparison to other investigations of nutritional intake during military training courses, the present study showed that EI (CAMP: 3420, FEX: 2697 and MIX: 2709 kcal·d^{-1}) was similar to that seen in Royal Navy and Royal Air Force OCs (2498 - 2677 kcal·d^{-1}) (Fallowfield et al., 2010). The data reported by Fallowfield et al. (2010) were used in 2016 to inform energy and macronutrient requirements of UK military personnel (SACN, 2016), especially for arduous occupational roles. Study 1 showed that although EI was low for all training settings, relative protein met the guidelines during camp training, which is essential to build and repair muscle tissue (Rodriguez et al., 2009). However, extremely low EI (1367 kcal·d^{-1}) was recorded during the field exercise and may cause concern given the importance of adequate energy for health and performance.

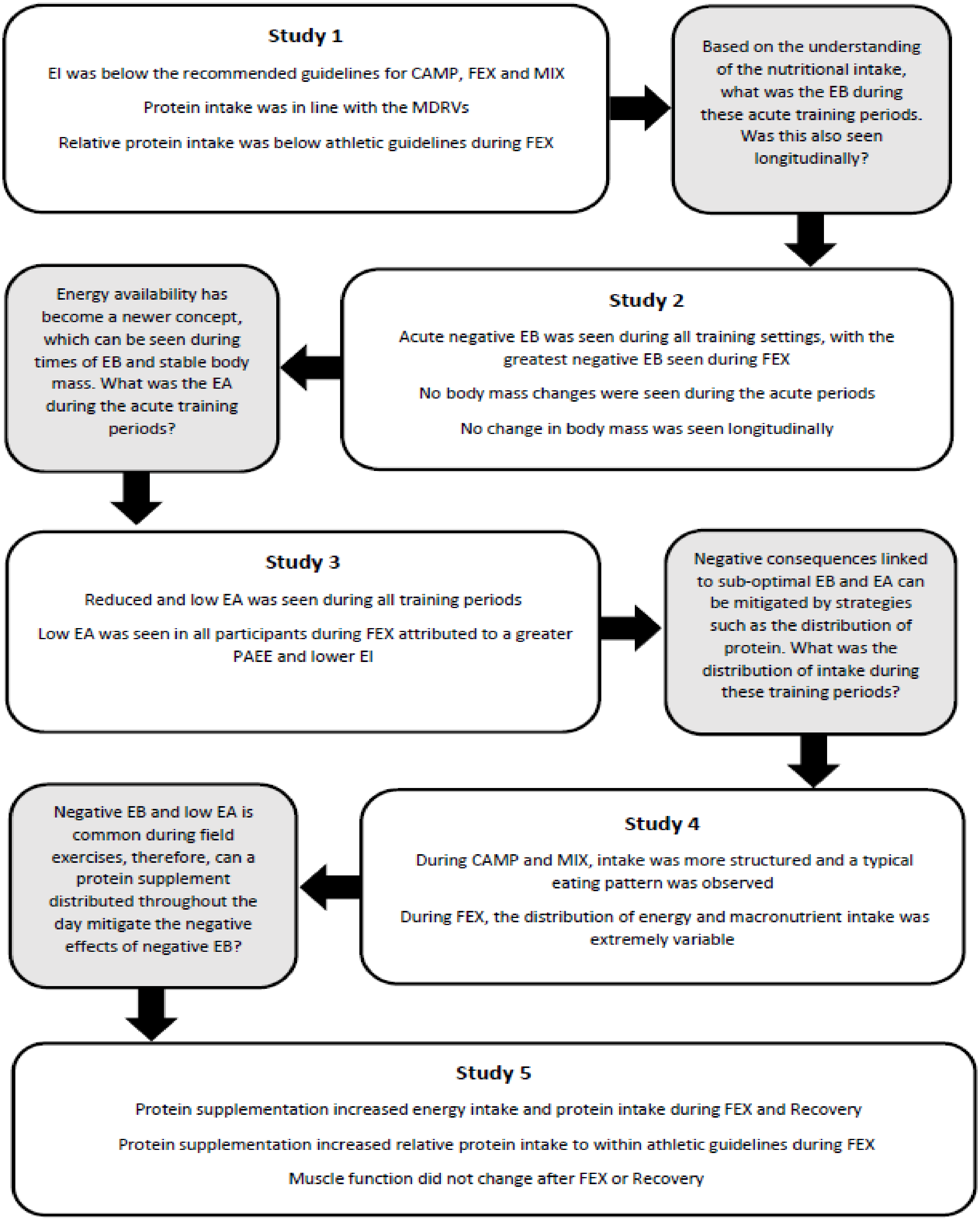

Figure 9.1: *Overview of Studies 1 to 5 within the thesis*

Low EI is not uncommon during field exercises when dietary intake is largely from ration packs. Although rations are designed to provide appropriate nutrition (Tassone & Baker, 2017), military personnel often have insufficient time to prepare meals to eat, and 'strip' items they do not want in order to save weight, and space, to prioritise combat equipment (Tassone & Baker, 2017). The lower intake during this period was also accompanied by a suboptimal carbohydrate and protein intake compared to athletic guidelines. However, despite the lower intake of these macronutrients, intake of fat remained relatively high. Although carbohydrate and protein are important for maintaining muscle mass (Calbet et al., 2017), recovering from exercise and fuelling exercise performance (Jeukendrup, 2008, 2010; Moore et al., 2014; Phillips & Van Loon, 2011), a higher intake of lipids can provide an energy potential twice that of carbohydrate and protein. Therefore, during FEX when OCs did not meet energy demands, a higher fat intake than the recommended guidelines may spare such high energy deficits.

In Study 2 the OCs body mass remained relatively stable over each of the three terms and year-long course, demonstrating a slight increase in body mass, and thus an overall Energy Balance (EB) in these periods. These results suggest that longitudinally, the overall demands of OC training are within the physical capabilities of most OCs based on the maintenance of EB, where OCs are able to offset periods of large energy deficits (spikes such as that seen in Week 8) by interludes of positive EB, resulting in maintained EB over time.

Although OCs were in a stable EB over the whole course, it was clear from Study 1, that there are multiple periods of high EE which may elicit acute periods of negative EB thoughout the year. Therefore, Study 2 quantified EB during the same training settings that had been assessed in Study 1 by quantifying EI and EE to calculate EB, as well as exploring the change in body mass over these acute periods. The approach of quantifying EB, using dietary records to measure EI and Doubly Labelled Water (DLW) to measure EE, showed that OCs were in negative EB during all three training periods, with FEX demonstrating the largest energy deficit. This is also not uncommon within military training, and it is often the case that training courses are designed to elicit military specific adaptations, used to better train and select appropriate personnel for mission requirements based on the scenario of extreme decrements (Tharion et al., 2005). However, a balance is required between the point where negative EB can be tolerated without any harmful consequences to health and performance. Based on the slope equation by Murphy et al. (2018) to predict decrements in performance and power, there would have been a zero to small (2%) decline in performance based on the total energy deficit of ~11,000 kcal for the whole of FEX reported in Study 2.

The lack of body mass changes that would have been expected to have accompanied such a large energy deficit in Study 2, was surprising. Previous published literature reviews have suggested that any energy deficit under three days is most likely water loss (Tassone & Baker, 2017), however longer training exercises between 8 to 12 days, which elicit a large energy deficit per day (-2150 to -2400 $kcal \cdot d^{-1}$), can result in decreases in body mass, Fat Mass (FM) and Fat Free Mass (FFM) (Alemany et al., 2008; Nindl et al., 2007). It may have been that the period in which participants were in an energy deficit (5 days) was too small to elicit any noticeable body mass loss. It could be argued that the difference in estimated and actual body mass changes were due to an underestimation of EI. However, the combination of wrapper collection and food weighing to measure EI offered the most practical and comprehensive solution for trying to ensure all dietary intake was captured across all sampling periods in field-based trials. Previous validation studies on self-reported dietary intake from ration packs in military personnel have demonstrated an agreement of > 95% with DLW (DeLany et al., 1989). Therefore, a more plausible explanation for the differences in estimated and actual body mass change are measurement errors from not measuring body mass at the same time of day or in the same clothing. Differences in conditions for body mass data collection was especially evident during FEX, where body mass was not measured immediately after the participants arrived back into camp, and therefore participants may have had the opportunity to 're-feed' and hydrate themselves prior to measurements, potentially explaining the lack of body mass changes seen during FEX.

Although EB has been the usual model for research and practice into energy requirements, research into Energy Availability (EA) has become more prevalent in the literature in recent years. Previous research has demonstrated that EB can be achieved in an energy-deficient state, without body mass changes, albeit with an accompanying suppression of physiological and metabolic processes (Loucks et al., 2011; Stubbs et al., 2004). Therefore, in military settings, it must be noted that a stable body weight, especially during times of arduous training, may not be an appropriate indicator of optimal physiological functions.

Study 3 demonstrated that all participants were in a low EA during FEX. This level of EA has been reported to disrupt and / or impair physiological, metabolic, and functional characteristics, including, but not limited to, metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Mountjoy et al., 2015). Therefore, simply quantifying changes in body mass may not be a true reflection of EA and its physiological consequences. Due to the limited research in EA, it is unknown at what magnitude or time period effects on physiological function would manifest, and further research in this area is required.

Taken together, studies 1, 2 and 3 demonstrated that during times of field exercise, OCs were in large negative EB and reduced or low EA, which was accompanied by a suboptimal intake of carbohydrate and protein. Based on recent literature that distributing protein throughout the day may be a more effective method to stimulate Muscle Protein Synthesis (MPS) compared to higher protein intake at fewer time points (Mamerow et al., 2014), optimising the timing of protein intake may be an effective strategy for military personnel.

Study 4 explored the distribution of energy, carbohydrate and protein intake, as well as hourly physical activity profile, during the same data collection periods as Studies 1 to 3. When OCs were able to access the dining hall or a field kitchen, food intake was distributed primarily around the core meals. During these periods of higher EI (*i.e.,* the core meal times), physical activity was typically lower when OCs were seated in the dining hall, demonstrating that OCs were able to rest and recover between periods of higher EE. This finding was also demonstrated in the evening, where OCs typically had their largest meal before a period of overnight rest. More interestingly, the key finding in Study 4 was the sporadic nature of food consumption throughout the field exercise, especially as physical activity was shown to be sustained low-to-moderate intensity for the duration of the field exercise, including overnight. The distribution of food intake during this period may reduce optimal recovery for continued low-to-moderate work especially given that total EI was not sufficient to maintain EB and resulted in reduced or low EA.

Study 1 detailed the physical activity profile over the CC, which demonstrated a spike in EE during week 8 of Junior Term. This time point was identified as an exercise named 'Exercise Longreach' in the Brecon Beacons, that is considered the most physically demanding exercise of the CC. The results from Study 1 demonstrated Exercise Longreach had a reported EE as high as 8200 $kcal \cdot d^{-1}$, as well as demonstrating that OCs were typically in negative EB and low EA during these periods, whilst having a suboptimal distribution of macronutrients. These results informed the design of Study 5 which aimed to examine the effect of a protein-rich dietary supplement during Exercise Longreach. The proposed benefit of increasing total dietary protein intake more evenly throughout the day during periods of large energy deficits, would be thought to preserve protein balance and support recovery from Exercise-Induced Muscle Damage (EIMD) (Margolis et al., 2016). It was also hypothesised that an increased protein intake during a field exercise, would reduce any decrease in performance and promote a greater recovery in the days following the energy deficit. The key findings in Study 5 were that a greater EI and protein intake was achieved by providing the protein supplement, which reduced negative EB. Despite the reduction of EB in SUP compared to CON, there were no differences in EA. Although it is unlikely that the short exercise period in this study would have elicited any physiological harm, it still emphasises the difficulty in optimising military feeding during field exercises, demonstrating the need to increase EI even further during longer duration field exercises, so that physiological, metabolic, and functional characteristics are not disrupted.

The results of Study 5 also demonstrated that no performance decrements occurred following the arduous exercise for either the control or supplement group. The lack of performance changes was surprising, as it was thought that decrements would have been present owing to the prolonged, unaccustomed exercise involving long periods of downhill walking whilst carrying heavy loads. This type of activity would normally be expected to cause reductions in force-producing capability associated with EIMD (Howatson & Van Someren, 2008). However, EIMD may still have occurred as indicated by the subjective measures of soreness and fatigue, which have been shown to be associated with EIMD (Howatson & Van Someren, 2008). The results in Study 5, are line with previous literature (Murphy et al., 2018; Pasiakos & Margolis, 2017) whereby the total energy deficit over the 2-day period (-9100 kcal) were below the limit to elicit lower body performance. However, although previous studies have demonstrated varying results after periods of energy deficits and arduous exercises, it is likely that the performance differences between these studies and Study 5 are due to the differences in cofounding variables such as environmental conditions (Margolis et al., 2016), the type of exercise performed (Millet & Lepers, 2004), and the magnitude of the energy deficit (Murphy et al., 2018).

Although no performance decrements were reported in Study 5, the possibility of impaired physiological functions, due to the low EA, cannot be ruled out. Hamarsland et al. (2018) measured performance in Norwegian soldiers undergoing a week of energy restriction, sustained physical activity, and sleep deprivation, which included near continuous marching over a hilly area with a 35 kg load. The study demonstrated a reduction in body mass, hormonal markers such as testosterone, and performance measures such as counter movement jump and maximal isometric strength performance. Interestingly, despite muscle mass and testosterone levels returning to normal after one week, performance did not. Therefore, Hamarsland and colleagues speculated that the mechanism of reduced performance was not due to these reduced factors. It can be speculated, that although performance was not reduced in Study 5, hormonal changes and other metabolic markers may have still been affected. Therefore, further research to understand the effects of low EA on physiological functions such as protein synthesis, hormone concentrations and performance, is warranted.

In hindsight, it is recognised that a longer duration study may have been better to determine the impact of the protein-rich supplement on performance. The exercise chosen for Study 5, was demonstrated to be the most arduous exercise of the CC, shown in Study 1, and therefore was thought to elicit the largest EB. It may have been likely that the other field exercises on the course would have been more suitable, as they would have elicited a similar negative EB, whilst also being longer in duration. However, Longreach, a load carriage exercise where OCs are required to walk a set distance with a controlled load, could have arguably been the most feasible exercise to elicit the most control compared to other exercises on the CC due OCs walking the same distance, carrying the same load and being supplied the same food.

Distribution of energy intake was not reported in Study 5 due to lack of adherence to reporting time of intake. Therefore, despite the participants stating that the supplement was consumed between main meals, the distribution of all food intake as well as the supplement was unknown. As shown in Study 4, it was unclear at what the time points meals were consumed on FEX, due to eating when time allows, as well as lack of sleep and structure of the day. Therefore, it is speculated in Study 5, where participants were active between 24 to 36 hours with lack of sleep, that this may also have been the case. It is likely that protein intake distributed evenly throughout the day would optimally stimulate MPS and preserve FFM, and therefore should be researched within future studies.

In Study 5 the lack of differences between CON and SUP may be due to the high protein intake already being consumed. During Recovery, CON were consuming 2.1 $g \cdot kg \cdot d^{-1}$, which is higher than the MDRVs. Similarly Pasiakos, Cao, et al. (2013) demonstrated that consuming protein beyond the RDA did not elicit any further influence on MPS, therefore it may be suggested that a plateau may exist, above which consuming more dietary protein confers no additional benefit to recovery and muscular performance. The already adequate protein intake may be due to increased awareness and knowledge around nutrition, and as previously seen in Study 1, habitual protein was already adequate in OCs during in camp training. Despite this, protein intake during times of exercise, within Study 1 as well as Study 5, did not meet the guidelines. Therefore, it may be possible that developing a tailorable education programme that recognises the realities of inadequate intake during exercise, may be able to provide more effective ‘field-stripping’ strategies to soldiers (Pasiakos & Margolis, 2017), than trying to implement supplementation alone.

9.1 Discussion of Methodological Approach

The methods used in the thesis have increased the understanding of nutritional intake and physical activity of OCs during training. Although field-based measures do not have the same level of control as laboratory-based measures, the techniques used in this thesis are valid, reliable and practical for use in similar future studies.

All studies in this thesis were conducted during military training to measure the impacts of daily training as closely as possible, and therefore, were designed to not conflict with the OCs daily schedule or impose undue burden on them. Due to the structure and the nature of military training, where OCs have a set schedule that usually is not changed, the level of control is comparable to some laboratory environments. The structured training, which includes structured field exercises, demonstrates a high level of control, not only in the physical activity, but also the nutrition and food available to OCs, as well as the hours of sleep and recovery. Although extreme alterations in these variables can be seen, especially during field exercises, this is most likely to have the same structure within each CC. Therefore, the energy deficits seen within the field exercises, will most likely be seen year after year. These similarities over the CC have been demonstrated in the CC in 2017 (Study 3), where the EE data reported was similar to OCs in Bilzon et al. (2006) and the longitudinal body mass data reported in the OCs were similar to the OCs undertaking the CC in 1997 (Harwood et al., 1999). Likewise, the EI and EE, and thus EB, reported in the 2017 cohort (Study 1 - 4) and the 2019 cohort (Study 5) at RMAS, were also remarkably similar. The data reported, can therefore give future OCs at RMAS invaluable information in the demands of the CC, as well as guidance in the recommended dietary intake needed to meet those demands.

Similarly, to the training schedule, the catering schedule in the dining facilities, are set on a weekly rota. When on exercise, all OCs are issued with ration packs that contain the same energy and macronutrients, which may demonstrate a greater level of control in comparison to free-living studies. It must be noted that Cadets, after 6 weeks of initial training, are free to purchase their own food, and therefore may limit the control.

As previously discussed in Section 2.5 in Chapter 2, EA has become prevalent in sporting research, however, there are currently no clear guidelines on the methods for undertaking an EA assessment (*e.g.,* the number of collection days, methodologies for assessing EI, Physical Activity EE (PAEE), or FFM). Only few studies have employed direct observational measurements of EI and PAEE, to calculate EA (Ong & Brownlee, 2017; Silva & Paiva, 2014; Viner et al., 2015), however methods of measuring EI and PAEE vary. The use of direct observation of nutritional intake, as well as using a 'research-grade' accelerometer to quantify EE, which has previously been validated by Siddall, Powell, et al. (2019), demonstrates the most accurate methods to predict EA. It must be noted however, that although the equation to quantify EA is standardised (Nattiv et al., 2007), this does not accurately depict the bodies true physiological state and can therefore be used as merely a prediction.

It is likely, that the acute lack of body mass changes in the Study 2, may have been due to hydration status as well as the time in which body mass measurements were taken. Although the researchers aimed to control for the timing and clothing worn, such as always recording body mass at the same time of data collection and weighing clothing to account for the added mass, errors in the measurements were occasionally unavoidable, such that body mass was weighed in a different set of clothing (*i.e.,* physical training kit vs. fatigues), or after eating.

The use of both DLW and tri-axial accelerometery has given a unique insight into the physical activity profile of the whole CC, as well as detailed evaluation of acute periods throughout the course. Doubly Labelled Water is the gold-standard method of measuring EE in free-living settings, and therefore provides valuable information on the physical demands associated with the CC. However, despite the accuracy and validity of DLW, the technique provides a mean estimate of EE over a series of days so cannot provide daily or minute-by-minute EE data. This was demonstrated in Study 2, where DLW was measured over 10 days, however FEX was only recorded over 5 days. It was likely that during this period that EE was underestimated. As well as using DLW to quantify overall EE, the use of the tri-axial accelerometer has shown the best agreement with DLW compared to other methods in the military setting (Siddall, Powell, et al., 2019), and therefore was proven to be an effective tool for measuring physical activity on an hourly, daily and weekly basis.

Dietary intake methods have, and will, continue to be a challenge for researchers within non-laboratory-based settings. As contemporary assessment of EI is known to be problematic (Gittelsohn et al., 1994), direct observation and nutritional weighing was used to limit the inaccuracy that may be seen in other retrospective methods. Although this method is described as the 'gold-standard' for nutritional analysis, it is not without its limitations. Firstly, although all food in the dining hall was weighed for dietary analysis, pre-made meals with multiple ingredients were weighed as 'one item'. Therefore, unless the exact ingredients were supplied by the chefs to the researcher, it was impossible to quantify the exact food items eaten. Even so, if this was known to the researcher, the exact ingredients in each portion, may vary from person-to-person. Secondly, it is possible that the burden of weighing food influenced participants' behaviour and eating habits (such as reduced intake in the dining facility), and therefore may not have accurately represented their usual intake.

The use of zip-lock bags and collection of all wrappers allowed the researchers to capture all food intake during the field exercises. In military environments, discarding of litter, including food wrappers, is forbidden, and therefore OCs must carry all rubbish with them. In all studies, all food wrappers were collected from OCs, which likely limited this underestimation of food consumption, as there was opportunity for all food items to be accounted for. During periods where OCs did not eat in the dining hall in Studies 1 to 4, and during periods where OCs were in camp for Study 5, food diaries were used. It is understood that food diaries tend to underestimate dietary intake by 9 to 30% (Hill & Davies, 2001; Tharion et al., 2004), and therefore there may be a level of inaccuracy in this measurement. In order to limit the inaccuracy within this measurement, two methods were employed; Firstly, researchers examined each food diary on collection and asked the participant on any unknown items. This allowed participants to recall any items they may have missed and quantify the size of portions that the participant had recorded. Secondly, during nutritional analysis, participant's daily food diaries were assessed based on their level of compliance, and any food diaries deemed to be 'abnormal' were questioned by the researchers. This has been considered a more accurate method because the researcher probes for items that the subject may have forgotten to record (Schoeller et al., 1990). If a participant had fewer than 70% compliance of food dairies per day, their data set was removed. Several of these factors could be improved upon in future studies, however in the context of the current studies, this was simply not feasible without further disruption of the participant's lifestyle and training.

As dietary weighing is slow and labour intensive, other dietary assessment methods were explored within the work of the thesis. This included the use of digital photography method (McClung et al., 2017), and the 'Snap-n-Send' method designed by Costello et al. (2017). Although these methods were quick and accurate in a University canteen environment, they were unfeasible within the military setting. The digital photography method required reference portions to be pre-weighed, and a digital camera to be positioned in the dining hall to capture the food portion size of each participant's plate. Although previous work was conducted in a military environment (McClung et al., 2017), it was difficult for researchers in the current work to quantify the exact items on the plate, due to food items being 'piled' on top of other items. Although the Snap-n-Send method, which required OCs to take photos of their food and send it via 'Whatsapp' to the researchers, has been demonstrated to be accurate within an athlete population (Costello et al., 2017), OCs are refrained from having any mobile phone devices upon their person, and therefore was impractical. Further studies, within various military establishments, are needed for the development in accurately quantifying dietary intake, such as a 'self-weighing' method which would rely on participants weighing each food item and recording it digitally on a mobile device or tablet that were located in the dining facilities.

Although there were limitations in the work conducted in the thesis, there were also many strengths that made the work unique and valuable to future research. The use of the 'gold-standard' techniques, such as dietary weighing and DLW, as well as using other valid tools such as the tri-axial accelerometer, gave an accurate understanding of the acute demands of the CC, and the longitudinal nature, was advantageous in understanding the longitudinal demands. Unlike laboratory-based studies that often are conducted following pilot work, field studies, especially in the military, are limited to time and therefore often are conducted within a single data collection period. Therefore, the nature of the current work, gave the ability to adapt and improve any measures which were used, such as exploring different methods of dietary analysis.

9.2 Future Work and Recommendations

9.2.1 *Recommended Research*

In light of the results from all five studies as well as previous literature, it is still unknown whether large energy deficits during short periods of arduous exercise have any physiological effect on soldiers, and whether a supplement or nutritional strategies can mitigate any negative consequences that may be caused. Study 1 builds upon previous literature that quantifies the nutritional intake of soldiers in training and demonstrated that despite being supplied adequate nutrition during camp and field-based training, EI may still be inadequate due to limited time to eat. Study 2 further demonstrated that the nutritional intake of the OCs was insufficient to meet the required nutritional demands placed upon the soldiers, which may lead to physiological consequences described in Study 3. The distribution of intake was also shown to be inefficient to optimise MPS and recovery when in training, as described in Study 4. Study 5 demonstrated that a higher protein intake during arduous exercise, did not reduce negative EB, but did improve overall protein intake. Therefore, taken together, the studies in this thesis can not only help to inform military personnel on the benefits of adequate nutrition whilst training but also to further explore nutritional strategies within the military setting.

With EA becoming more prevalent within literature, it is recommended that future studies explore the physiological effects, such as hormonal health, bone health, Resting Metabolic Rate (RMR) and metabolic health, within the military environment during times of energy deficits. This research should look to explore the daily changes in RMR and hormonal markers, over short and longitudinal periods of low and / or reduced EA, as well as exploring 're-feed' periods to understand the time-frame in which RMR and hormonal changes return to baseline. This should incorporate controlled laboratory studies, where EE, EI and PAEE are kept standardised, as well as field studies, where these variables are interchangeable. It is also recommended that the time-frame and magnitude of these periods are explored, using varying length duration studies, as well as varying energy deficits, to gain a greater understanding of how this may affect soldiers in shorter and longer duration negative EB.

It is also recommended that whole-body protein turnover be explored within these periods of large energy deficits as well as during recovery periods within training, to determine what effect short and long-term negative EB may have on their training. This research should aim to explore, in both laboratory and field environments, the varying length and magnitude of an energy deficit on protein turnover and body mass changes, to determine at what point an energy deficit can be tolerated without any harm to health or performance.

Finally, to better understand the role of macronutrient distribution in optimising nutritional intake, further work is warranted in both a controlled laboratory and field environment to explore how distributing macronutrient, especially protein, intake throughout the day, can affect MPS and Muscle Protein Breakdown (MPB) during negative EB and low EA.

These recommendations may also help to inform further work to assess optimal nutritional strategies for optimising food consumption when in field-based environments, in order to enhance and promote physiological sustainment and recovery.

9.2.2 *Applied Recommendations to the Military*

This body of work provides military personnel with research, evidence and guidance on nutritional practice during times of training and arduous field exercise. The work can be used to inform military personnel on nutritional best practices and provide recommendations to optimise nutrition, benefiting performance, health and recovery.

Based on the body of work that has been conducted in this thesis, the following recommendations are offered to those that are undergoing, and / or teaching those that are undergoing, arduous training and field exercise / operations;

1. Military dietary reference values should be adhered to by military personal during all training courses when undergoing camp-based training. During field exercises, military personnel should ensure energy intake meets or exceeds the military guidelines and that any rations ‘stripped’ are replaced with other supplementary items to meet the required calorie need.

2. To ensure optimal intake during and after arduous exercise, for the benefit of maintaining and / or improving training adaptation and recovery, military personnel should consume nutrient rich food throughout a field exercise period where possible, ensuring a high protein and carbohydrate intake. Protein intake should be above the military guidelines and in line

with athletic guidelines of at least 1.2 $g \cdot kg \cdot d^{-1}$ and carbohydrate intake should be above the military guidelines and in line with athletic guidelines of at least 6 $g \cdot kg \cdot d^{-1}$.

3. To ensure optimal stimulation of muscle protein synthesis in order to aid recovery and adaptation, dietary intake should be spread evenly throughout the day. Protein and carbohydrate should be consumed during each meal and soldiers should aim to consume nutrient rich food before, during and after exercise where possible.

4. During acute periods of arduous exercise when time is limited to eat and energy requirements are difficult to meet with energy intake, 'easy to eat' items with a higher fat intake would be recommended, supplementary to the usual dietary intake, to mitigate any possibility of negative energy balance.

5. In addition to ensuring optimal nutrition during training and field / operational exercise, military personnel should ensure a higher energy intake with nutrient rich food is consumed during the following days post-field exercise to accelerate restoration of energy balance and energy availability.

6. To help evaluate their own or their student's energy balance acutely and longitudinally, military personnel should employee methods of measuring body mass and composition on a regular basis. By understanding the magnitude of energy balance as well as the risks associated, it may allow personnel to optimally adapt and recover from training as well as mitigate the risk of performance decrement and injury.

7. Military personnel should take precautionary measures to ensure both themselves and their staff / students are mitigating the risks of low energy availability where possible. This should include; awareness and education to all military personnel on low energy availability and the consequences that this may entail. The use of screening techniques such as the 'Low Energy Availability in Females Questionnaire' to assess the risk of low energy availability, and were possible, routine medical examination of military personnel are recommended to evaluate symptoms of any energy deficiency's and where possible be paired with biochemical, clinical and hormonal tests to attribute the findings of impaired function to low energy availability.

9.3 Conclusion

The research presented in this thesis demonstrates that the British Army CC results in high levels of EE and dietary intake of some OCs did not meet current military dietary guidelines, typically due to the high sustained EE and limited time to eat. The OCs body mass remained stable during the course which indicates that the OCs were in EB. However, during acute periods of arduous activity during the course the OCs experienced periods of sustained high EE when they are required to be physically active during the night and day. The mismatch in EE and EI resulted in negative EB during these periods as well as reduced or low EA which can lead to disruption and / or impaired physiological and metabolic function. Nutritional strategies such as the distribution of protein around physical activity may help mitigate the negative consequences associated with negative EB. However, the distribution of dietary intake during FEX was highly variable both within- and between- participants. Consuming a dietary protein supplement twice-a-day during an extremely arduous field exercise on the CC increased OCs EI and macronutrient intake. However, this had no effect on the measurements of EIMD following the field exercise. Further research is required to investigate whether a protein rich supplement would affect other detrimental consequences associated with negative EB and low EA such as hormonal health, bone health, RMR and metabolic health within the military environment.

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Appendix A

Comparison of Body Composition measured via DXA and TBW

Introduction

As part of the larger project within the thesis, pilot work was conducted to determine the agreement of body composition data from Duel Energy X-ray Absorptiometry (DXA) and Total Body Water (TBW), to determine whether TBW can be used as a valid measure of body composition within the scope of the thesis. The aim of the work, was to determine the agreement in Fat Mass (FM) and Fat Free Mass (FFM) between DXA and TBW.

Method

Participants

Twenty participants (mean ± SD: men: age 24 ± 2 years, height 1.79 ± 0.06 m, body mass 86.1 ± 6.6 kg; women: age 24 ± 3, height 1.68 ± 0.04 m, body mass 71.7 ± 3.7 kg) volunteered to take part in the study Ethical approval was granted by the Ministry of Defence Research Ethics Committee (protocol 931/MoDREC/18). Participants were provided with a verbal and written brief on the requirements as well as any possible side effects of the study and were offered the opportunity to ask questions before providing informed written consent.

Study Design

Body composition was estimated through two methods; TBW, calculated through DLW, and DXA. On day 0, DLW was administered before 10 further days of urine samples were collected, followed by two non-training days (day 11 and 12). Body composition measurements using DXA were taken on the next available training (day 13) before lunch, described in Figure 11.1.

Doubly Labelled Water

The dosing of DLW is described in Section 4.3.5 in Chapter 5. Body composition was estimated through TBW, which is described in Section 3.3.1 in Chapter 3.

Duel-energy X-ray Absorptiometry

To measure body composition via DXA (GE Lunar iDXA, GE Healthcare, Chalfont St Giles, UK), participants were required to wear minimal clothing (shorts and t-shirt, no shoes), remove all metal items and to state whether they were pregnant. A trained operator performed all scans according to the manufactures guidelines in operating the machine and positioning of the participant. Further details of the method are described in Section 3.3.2 in Chapter 3.

Data Analysis

Statistical analysis was run on Statistical Package for the Social Sciences (SPSS), where FM and FFM, using the two methods were correlated using a Pearson's Correlation Co-efficient, and the differences between each method were compared with a one-sample t-test. A result of no difference between each method and a correlation would be further assessed for agreement using a Bland-Altman plot to compare means. Statistical significance was set *a priori* at $p<0.05$.

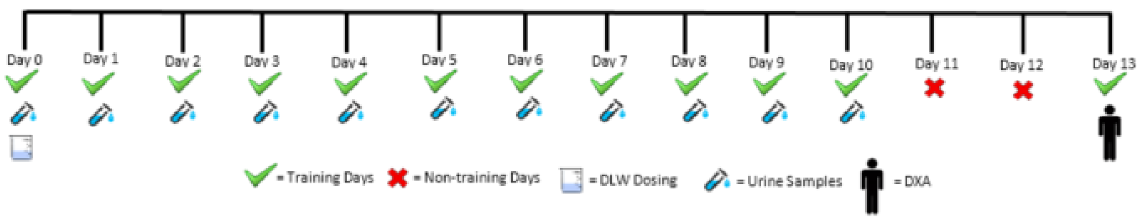

Figure 11.1: *Schematic of the study design demonstrating the time period where Doubly Labelled Water (DLW) was dosed and Duel-energy X-ray Absorptiometry (DXA) was measured to estimate Fat Mass (FM) and Fat Free Mass (FFM)*

Results

There was no difference between FM measured by TBW and DXA (t (19) = 0.592, p = 0.592, d = 0.122), however there was a correlation (r = 0.56; p = 0.11). The Bland-Altman was used to plot the differences in FM for each participant against the mean of the two measurements (Figure 11.2A), with a mean bias of 0.5 ± 4.4 kg, where 95% of the difference lay between -8.1 and +9.1 kg.

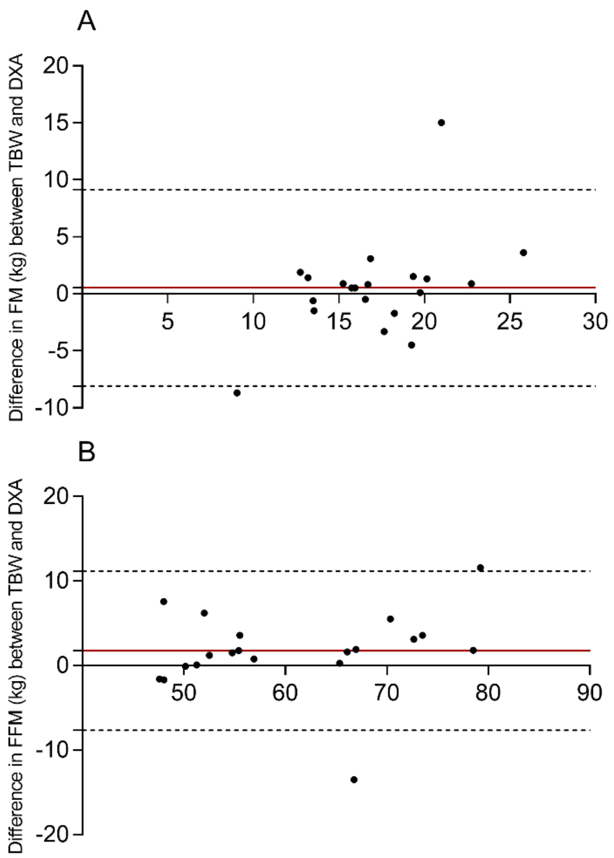

Figure 11.2: *Bland-Altman plot of agreement [mean difference (solid line) ± 95% Limits of Agreement (dashed line)] between Fat Mass (FM; A) and Fat Free Mass (FFM; B) as calculated by Total Body Water (TBW) and Duel-Energy X-Ray Absorptiometry (DXA)*

There was no difference between FFM measured by DXA and TBW (t (19) = 1.642, p = 0.117, d = 0.367), however there was a correlation (r = 0.90; p < 0.001). The Bland-Altman was used to plot the differences in FFM for each participant against the mean of the two measurements (Figure 11.2B), with a mean bias of 1.8 ± 4.8 kg, where 95% of the difference lay between -7.6 and +11.2 kg.

Discussion

The pilot work presented was used to determine the agreement of body composition data from DXA and TBW to be used within the current body of work. The results demonstrated that there was a strong correlation between methods for FFM, and a moderate correlation for FM.

Similar to the present data, other studies have demonstrated DXA to underestimate the body fat of leaner individuals compared with other body composition methods such as underwater weighing, TBW, and bio-impedance analysis (Friedl et al., 1992; Fuller et al., 1992; Gallagher et al., 2000; Van Der Ploeg et al., 2003).

Both TBW and DXA techniques, to measure body composition, have advantages and disadvantages, where both methods are limited due to high cost and need for specialised equipment. The use of DXA is advantageous when determining the change in body composition over a set period of time, and can be relatively simple and quick method to determine FM and FFM. However, when DLW is already being employed, and when participants are limited on time, the use of DLW may be better suited.

Limitations within the present pilot study may have varied the results, such as the time of day the measurements were taken and the control in hydration. For instance, due to nature of the participant's workload, control of intake of food and water was not accounted for, therefore participants may have consumed any food or drink between the two measurements that could have affected the data. DEXA assumes a hydration of fat free mass to be approximately 73%, therefore acute changes in hydration may have affected the measurement (Esco et al., 2017). This has been demonstrated in previous work, that demonstrated DXA to significantly overestimated tissue mass towards the end of an 8-week course, which is speculated to be due to a misinterpretation of hydration assumptions (Friedl et al., 1994). Likewise, TBW assumes the hydration of FFM to be 73% and to be relatively constant (Westerterp, 2017). However this exact value of a healthy adult can reportedly fluctuate ± 5% daily, due to ongoing physiological processes and the consumption of food and drink (Chumlea et al., 1999), which may be even more so in the likes of military establishments. Therefore, both methods should be reported with caution.

The present data exhibited good validity between FM and FFM measured via TBW compared to DXA, and thus suggest that TBW can be used as a valid measure of body composition within the scope of the main thesis.