Energy Intake and Appetite Following Sport-Specific Exercise in Adolescent Girls

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ABSTRACT

The overall aim of this thesis was to establish whether free-living energy intake, subjective appetite and objective appetite (acylated ghrelin concentrations) would be influenced by a bout of netball specific exercise in female adolescent netball players. As part of this thesis four studies were conducted, the first two were deemed methodological studies, whilst the third and fourth studies were deemed intervention studies. In chapter three a representative fitness test was established to assess exercise intensity and energy expenditure as accurately as possible in female adolescent netball players (13-15 years old). In addition, exercise and appetite studies require energy intake to be assessed as accurately as possible, since under-reporting of energy intake is an inherent problem in normal-weight adolescent girls of increasing age. Consequently, chapter four of this thesis investigated the agreement between observers recorded energy intake and energy intake from a combined self-reported, weighed food diary and 24-hour recall interview technique, in female adolescent netball players (14-16 years). The methods developed in chapters three and four, were then used in chapters five and six to investigate the relationship between exercise, energy intake and appetite. Thus in chapter five, energy intake and appetite following netball exercise over 5-days, in trained 13-15 year old netball players was explored. Findings from chapter five influenced the study discussed in chapter six, which employed a similar methodology to that of the study in chapter five. In addition however, chapter six explored hunger more objectively, by assessing concentrations of the orexigenic hormone, acylated ghrelin, following a bout of netball exercise in both trained and untrained adolescent girls. Overall, several key findings were identified in this thesis. Firstly, chapter five identified that immediately following a bout of netball exercise the girls felt significantly more hungry compared to immediately before the exercise bout. In addition, over 2-days (48-hours) following the netball exercise bout energy intake was significantly elevated when compared to a sedentary period. Secondly and finally, chapter six identified that untrained 13-15 year old girls undergoing intermittent netball exercise, increased their free-living energy intake 3-days (72-hours) following a bout of netball exercise when compared to a sedentary intervention. In addition, 20 minutes during the netball exercise a group of trained and untrained girls reported that they felt significantly more full compared to the corresponding time in a sedentary intervention. The last study (chapter six) was also the first study to explore acylated ghrelin concentrations following exercise, in trained and untrained adolescent girls.

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ATP	Adenosine Tri-Phosphate
BMI	Body Mass Index
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
MJ	Megajoule
NSEP	Netball specific exercise protocol
SED	Sedentary
VAS	Visual Analogue Scale
$\dot{V}CO_2$	Carbon Dioxide Production
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_{2\ max}$	Maximum Oxygen Uptake
$\dot{V}O_{2\ peak}$	Peak Oxygen Uptake

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AUTHOR DECLARATION

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work.

Name:

Signature:

Date:

CHAPTER 1

Introduction

The short-term relationship between exercise (and thus exercise-induced energy expenditure) and subsequent energy intake is presently not well defined in paediatric populations. The three short-term intervention studies thus far which have explored energy intake and appetite following exercise in paediatric populations have been conducted in young lean girls (9-10 years; 10-11 years) (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008), young overweight girls (10-11 years) (Dodd, Welsman & Armstrong, 2008) and lean adolescent girls (12-14 years) (Rumbold & Dodd, 2007). Knowledge and evidence surrounding the effect of exercise on energy intake and appetite in these populations appears to be unequivocal, with all of them concluding that the body does not crave nor require extra energy from food following a single exercise session and that in lean girls there is no automatic increase in hunger (Moore *et al*., 2004: Rumbold & Dodd, 2007: Dodd, Welsman & Armstrong, 2008). However, especially with regards to lean adolescent girls, who have a positive maturity offset, there is a requirement to maintain energy balance following exercise, by increasing their energy intake, since such a marked negative energy balance would result in continual weight loss. Therefore, it is of interest to explore the energy intake and appetite responses of adolescent girls more fully.

Laboratory-based exercise protocols have been the prefered choice of exercise intervention in two of the cited paediatric studies (Moore *et al*., 2004: Dodd, Welsman & Armstrong, 2008), however laboratory-based exercise protocols lack specificity as they fail to represent the habitual exercise patterns of young people. The sport specific short-term intervention studies which have employed appropriate free-living exercise interventions have mainly been conducted in adult groups, with only one previous study of adolescent girls (Rumbold & Dodd, 2007). There is therefore now a need for paediatric studies to adopt the approach of Rumbold and Dodd (2007) and move forward from laboratory based exercise interventions, to the use of more representative or free-living exercise protocols in short-term exercise and appetite studies. With regards to the free-living exercise interventions in the adult studies, at present these only include athletic activities (not specified in the studies) (Verger, Lanteaume & Louis-Sylvestre, 1992; 1994) and swimming (Verger *et al*., 1992). Findings from the three cited studies have been inconsistent with regards to the energy intake responses following the exercise bout, possibly due to methodologies lacking precision in terms of energy intake and energy expenditure assessment techniques. Therefore, there is a need for robust methods to be developed, with regards to energy expenditure and energy intake assessement, to ensure that data in terms of these parameters can be collected as accurately as possible, especially in paediatric populations.

In one study of adults, when research was extended into the free-living environment using self-reported, weighed food diaries, partial energy intake compensation for exercise was observed. This study involved six lean women completing seven consecutive days of high intensity cycling (3 x 40-minutes / 3.4 $MJ \cdot day^{-1}$), which resulted in partial energy intake compensation (~30%) when diet was unrestricted (free-living) (Stubbs *et al.*, 2002b). Unfortunately, there is no comparable literature from paediatric groups with regards to free-living energy intake using a robust energy intake assessment technique, following exercise over several consecutive days. As discussed previously, only three short-term intervention studies to date have investigated the relationship between energy intake and exercise-induced energy expenditure in young people (Moore *et al.*, 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008), all of which monitored laboratory based food intake following the prescribed exercise through observation and the weighing of *ad libitum* food intake.

These studies demonstrated that either within 1 hour of exercise cessation (Rumbold & Dodd, 2007), over the course of 1 day (Moore *et al.*, 2004), or over the course of 5-days (Dodd, Welsman & Armstrong, 2008) there was no elevation in energy intake following an acute exercise bout when compared to a sedentary condition. Moore et al. (2004) state that this work should now be extended into the free-living environment, where young people control what and when they eat.

In addition, appetite parameters such as hunger, fullness and prospective food consumption are typically explored alongside energy intake in exercise and appetite studies. It has been suggested that high-intensity exercise can result in a short-term suppression in appetite (exercise-induced anorexia) (Blundell & King, 1999). This appetite suppression appears to be dependent on the intensity of the imposed exercise with an optimal exercise intensity of 75% $\dot{V}O_{2\,max}$ inducing maximal appetite suppression (Thompson, Wolfe & Eikelboom, 1988; King, Burley & Blundell, 1994). King, Burley and Blundell (1994) identified that appetite is suppressed for up to 15 minutes after exercise primarily in adult males but this has not been consistently identified in adult females (King *et al.*, 1996). With regards to paediatric groups, the first published study conducted by Moore et al. (2004) examining energy intake and appetite responses to exercise, found that 9-10 year old girls cycling at 75% $\dot{V}O_{2\,peak}$ demonstrated a desire to eat less (p<0.01) immediately following the cycling bout compared to cycling at 50% $\dot{V}O_{2\,peak}$ and a sedentary condition, which was short-lived. In a similar high intensity exercise protocol, Dodd, Welsman and Armstrong (2008) identified that six overweight girls felt significantly hungrier compared to six lean girls (11.5±0.4 years) immediately post exercise. This was not reflected however by any subsequent increase in energy intake. Laboratory-based protocols are a popular means of manipulating energy expenditure, however they lack specificity as they fail to represent the habitual exercise patterns of young people. It has been suggested that intermittent activities are more attractive and motivating to young people (Ratel *et al.*, 2004) increasing levels of conformity and participation (Livingstone, Robson & Totton, 2000). In schools a major element of the National Curriculum for Physical Education anchors teachers to deliver sporadic game-based activities, such as netball and football. Therefore, using sport-specific exercise, which is more representative of young girls activity patterns, Rumbold and Dodd (2007) found no differences between the appetite parameters (hunger, prospective food consumption and fullness) immediately post-conditions when intermittent netball based exercise was compared to a sedentary condition. Therefore, as there is no consistency in findings to date in paediatric groups, there is a necessity for further research into subjective appetite responses to exercise in young children and adolescents, which needs to move forward from laboratory based exercise interventions and use more representative or free-living exercise protocols.

It can also be suggested that because all of the appetite studies described above used subjective assessment, a more objective measure of hunger, such as hormone analysis, would help to clarify how young children and adolescents respond to exercise with regards to appetite. Interestingly, a recently discovered orexigenic hormone (appetite stimulant) known as ghrelin (Wren *et al*., 2001; Cummings, 2006) has been explored following exercise in adult populations to provide an objective measure of the neuro-physiological pathways associated with appetite. In addition, with regards to previous research concerning ghrelin in adult populations, there have been inconsistencies in findings, which have been dependent on whether the participants were classified as trained in terms of the exercise protocol used in the respective study or untrained and not familiar with the type of exercise imposed. Furthermore, there is no available data regarding short-term ghrelin responses to exercise in children and adolescents, in either trained or untrained population groups.

Consequently, it would be of interest to investigate: energy intake, energy expenditure, energy balance, subjective appetite and ghrelin (as an objective measure of ‘appetite’) in a free-living environment, following sport-specific exercise, whilst also directly comparing a group of trained and untrained adolescent girls.

1.1 Thesis Purpose and Aims

The purpose of this thesis was as follows. Chapter two provides a review of the available paediatric literature with regards to the physiological aspects of appetite and energy intake regulation, followed by an outline of assessment techniques used to measure subjective and objective appetite (hormone analysis), energy intake and energy expenditure in paediatric populations. Finally, an overview of short-term intervention studies with regards to exercise, energy intake and appetite in paediatric populations will be provided.

Following this, chapter three and chapter four respectively, will establish an appropriate method for assessing sport-specific exercise intensity and energy expenditure for the use with adolsecent populations in a free-living environment and also explore how accurate adolescent populations are at recording their own energy intake using a combined self-reported, weighed food diary and 24-hour recall interview technique. The establishment of these methods will ensure that the data collected in the subsequent two studies (chapters five and six) are as accurate as possible.

Chapter five will then employ the methods established in chapters three and four and intervene with a netball specific exercise protocol (NSEP) to explore free-living energy intake and subjective appetite over a 5-day period in a group of trained, female adolescent netball players (13-15 years). Chapter six will then follow a similar organisation to that of chapter five, however the study period will be extended over 7-days as opposed to 5-days. This study will also directly compare free-living energy intake, subjective appetite and objective appetite following a NSEP and sedentary (SED) intervention in a group of trained, female adolescent netball players and a group of untrained adolescent girls (13-15 years). In addition, even more sensitive techniques to that of chapter five with regards to the measurement of energy expenditure (simultaneous heart rate and physical activity data) and objective appetite (plasma acylated ghrelin concentrations) will be examined.

The final chapter, chapter seven, will then provide a synthesis, linking the results observed in chapters three to six to the literature presented in chapter two.

Therefore, the overall aim of this thesis was to establish whether free-living energy intake, subjective appetite and objective appetite (acylated ghrelin concentrations) would be influenced by a bout of netball specific exercise in female adolescent netball players.

CHAPTER 2

Literature Review

The following literature review will firstly outline some special considerations when working with a paediatric population. This will be followed by a review of the fundamental principles concerned with appetite and energy intake regulation, in particular the appetite hormone ghrelin. Energy intake and energy expenditure assessment techniques will then be reviewed, followed finally by findings pertaining to energy intake, subjective appetite and ghrelin responses to exercise. Throughout all of the review there will be a specific focus on paediatric populations, however where no pertinent information is available, literature from adult groups, in particular females where possible, will be examined.

2.1 Special Considerations for Working with Young People

Recent government initiatives such as the School Sports Strategy (Department for Culture, Media & Sport, 2007) propose that sport should be a part of every young person's school day. Physical activity and structured moderate intensity exercise are popular means of promoting health benefits (Valanou, Bamia & Trichopoulou, 2006) and increasing levels of energy expenditure. Physical activity performed by young people can occur in a variety of forms such as free play, house chores and school physical education (Malina, Bouchard & Bar-Or, 2004). Thus for the purpose of this thesis, adolescent girls who only participate in the forms of physical activity previously identified and are not habitually involved in additional extra-curricular school sport and exercise will be referred to as 'untrained'. Training for sport must not be confused with physical activity (Malina, Bouchard & Bar-Or, 2004), as it is defined as being above and beyond normal levels of physical activity (Baxter-Jones & Mundt, 2007). Adolescent girls involved in such specialised practice for a specific sport (primarily team sports) will therefore be referred to as 'trained', throughout this thesis.

Indeed, when working with all adolescent populations, regardless of physical activity level or training status, there are various special considerations which need to be accounted for. These include the assessment of biological maturity, body composition and aerobic fitness. The measurement of these variables is even more important when comparing groups of adolescents.

2.1.1 Controlling for Biological Maturity

Chronological age is often used as a point of reference in paediatric groups, although it must be recognised that individuals of the same chronological age are likely to differ in terms of biological maturity (Malina, Bouchard & Bar-Or, 2004; Baxter-Jones & Sherar, 2007). One year of chronological age does not equate to one year of maturational time (Baxter-Jones & Sherar, 2007). It is important therefore to control for biological maturity, as individuals of the same chronological age can be at different degrees of biological maturity, due to the variability in the timing and tempo of maturation between individuals (Malina, Bouchard & Bar-Or, 2004; Baxter-Jones & Sherar, 2007).

Age at peak height velocity is used most commonly as an indicator of maturity in young people (Baxter-Jones & Sherar, 2007). The maximal physical growth spurt is known as peak height velocity (Baxter-Jones & Sherar, 2007). The onset of peak height velocity occurs in girls occurs on average, between 8.2 and 10.3 years old, and is typically attained between 11.3 and 12.2 years old in boys (Baxter-Jones & Sherar, 2007). Age at peak height velocity can be calculated (Baxter-Jones & Sherar, 2007) or predicted (Mirwald *et al*., 2002), but either way it is a quick and non-invasive technique. Calculating age at peak height velocity is limited to longitudinal studies, where the same participants are assessed over time and serial measures of height and age are recorded over a number years (Mirwarld *et al*., 2002). Consequently, predicting age at peak height velocity is more appropriate for use in short-term intervention studies where an indication of maturity status is required in order to control for maturity (Mirwald *et al*., 2002). Mirwald et al. (2002) developed a simple, non-invasive technique, using anthropometric measures, to assess maturity, based on predicting years from peak height velocity (Mirwald *et al*., 2002). The cited authors developed sex-specific multiple regression equations, to predict years from peak height velocity, using the growth patterns of height, sitting height and leg length. Consequently, age at peak height velocity can be predicted by relating years from peak height velocity to current age (Baxter-Jones & Sherar, 2007). Various anthropometric measures are required such as stature, sitting height and leg length in addition to body mass and chronological age (Mirwald *et al*., 2002). For girls the prediction equation to identify maturity offset was established as (the term interaction refers to multiplication):

-9.376 + (0.0001882·leg length and sitting height interaction) + (0.0022·age and leg length interaction) + (0.005841·age and sitting height interaction) – (0.002658·age and weight interaction) + (0.07693·weight by height ratio) (Mirwald *et al*., 2002, p. 692).

The R^2 value was 0.890 and the standard error of estimate was 0.569, which indicated that 89% of the variance was accounted for by the prediction equation and the error in the prediction was 0.5 years (Mirwald *et al*., 2002). Thus 95% of the time age at peak height velocity can be predicted within ±1 year (Mirwald *et al*., 2002).

The maturity values obtained using the Mirwald et al. (2002) prediction equation can be used in research studies in two ways to describe participants. Firstly, in terms of maturity offset values, these provide the time lag in years, before or after the predicted height velocity of the individual. Therefore a negative maturity offset value would indicate that the individual had not reached peak height velocity and a positive maturity offset would imply that they had (Mirwald *et al*., 2002). Secondly, age categories can be based on adolescent girls being early, average and late maturers. Thus average maturers would be those whose predicted age at peak height velocity falls within ±1 year of the average age at peak height velocity (12 years for girls), whilst early and late maturers would have predicted age at peak height velocities which occur more than 1 year prior and more than 1 year, respectively, after the mean age (12 years old) (Baxter-Jones & Sherar, 2007).

A second special consideration when working with adolescent populations is the estimation of body composition.

2.1.2 Estimation of Body Composition

Estimation of body composition in energy intake and appetite research studies is important, especially in independent study design when two unrelated participant groups are being compared. Plethysmography is the measurement of size and in particular volume (Fields, Goran & McCrory, 2002) and is one way of estimating body composition. Air displacement plethysmography is the indirect measurement of an objects' volume by quantifying the volume of air it displaces in an enclosed chamber (plethysmograph). Therefore with regards to human body volume, when a participant sits inside the chamber a volume of air equal to their body volume is displaced (Fields, Goran & McCrory, 2002). The volume of air remaining in the chamber when the participant is sitting in it is subtracted from the volume of air contained in an empty chamber, thus providing an indirect estimate of body volume (Fields, Goran & McCrory, 2002). With specific regards to the Bod Pod (Dempster & Aitkens, 1995) (Life Measurement Instruments, Inc., Concord, CA), by measuring changes in pressure within the enclosed chamber body volume can be determined (Heyward & Wagner, 2004).

The Bod Pod allows body volume and from this body density and percentage body fat to be predicted in young people, using procedures which are quicker, safer, more convenient, easier to administer, more comfortable for participants and generally less invasive compared to hydrostatic weighing (Wagner & Heyward, 1999) and other body composition estimation techniques (Fields, Goran & McCory, 2000). Consequently, the Bod Pod is an appealing technique to researchers who work with paediatric populations. Indeed, the Bod Pod has been successfully used in several paediatric exercise and appetite studies to date involving younger girls (9-11 years old) (Moore *et al.*, 2004; Dodd, Welsman & Armstrong, 2008) and active adolescent girls (netball players) (12-14 years old) (Rumbold & Dodd, 2007).

With regards to the validity of the Bod Pod in comparison to reference techniques, in particular hydrostatic weighing, which is classified as the 'gold standard' technique for assessing fat mass and fat-free mass (2 compartment model), relatively few studies have been conducted, when estimating percentage body fat in adolescent girls. From the available validation studies in young people the results are equivocal. Nuñez et al. (1999), Dewit et al. (2000) and Wells et al. (2000) identified that estimates of percentage body fat made using the Bod Pod were higher compared to hydrostatic weighing, although not significantly different (0.6-1.7%). Lockner et al. (2000) also reported R^2 values of 0.87, indicating that the Bod Pod explained 87% of the variance in hydrostatic weighing. Fields and Goran (2000) also reported a good, standard error of estimate value for percentage body fat of 3.3%. Fields and Goran (2000) investigated the validity of the Bod Pod by regressing fat mass calculated by the Bod Pod with fat mass from dual energy X-ray absorptiometry, as oppose to percentage body fat. Consequently, it was concluded that the Bod Pod was an accurate and precise technique for estimating fat mass in 9-14 year old children (Fields & Goran, 2000), as long as methodological and biological factors are held constant.

In summary, it seems that in young people the Bod Pod is a superior method of body composition estimation in relation to field techniques, its accuracy is comparable to other laboratory techniques such as hydrostatic weighing. Since the Bod Pod is a practical and not a logistically demanding technique, it is consequently an appealing method to use especially with regards to exercise and appetite studies involving young people, where a measure of body composition is important.

2.1.3 Assessing Exercise Intensity and Ratings of Perceived Exertion

The last special consideration, with regards to this thesis, is the assessment of sport-specific fitness. Typically in adults $\dot{V}O_{2\,max}$ is assessed as an indicator of aerobic fitness (Welsman & Armstrong, 1996). $\dot{V}O_{2\,max}$ is where oxygen uptake increases linearly with progressive exercise until a point is reached and it plateaus, despite further increments in exercise intensity (Welsman & Armstrong, 1996). However, a plateau in oxygen uptake is not always established in young people, as less than 50% of young people attain a plateau in oxygen uptake during exercise tests to exhaustion (Rowland & Cunningham, 1992). Thus during a progressive exercise test to exhaustion in children and adolescents the term $\dot{V}O_{2\,peak}$ is used to denote the highest $\dot{V}O_2$.

Blood lactate is not accepted as a criterion to determine $\dot{V}O_{2\,peak}$ in young people (Armstrong & Fawkner, 2007). Armstrong and Fawkner (2007) state that validating $\dot{V}O_{2\,peak}$ in young people against post-exercise blood lactate values is flawed, due to the range of values obtained as a result of differences in the mode of exercise, exercise protocol, time of sampling, site of sampling, blood treatment and assay procedures.

Differences between the $\dot{V}O_{2\,peak}$ of untrained and trained young people are apparent, with research suggesting that the latter group have higher $\dot{V}O_{2\,peak}$ values (Rowland, 1990). The difference in $\dot{V}O_{2\,peak}$ values between trained and untrained populations may be explained by the recent data published in the Health Survey for England (2008) (Craig, Mindell & Hirani, 2009) which suggests that low levels of physical activity levels are apparent. The Health Survey for England (2008) concludes that the percentage of girls meeting the government recommendations for physical activity has decreased from 35% at age 2 years to 12% at age 14 years (Craig, Mindell & Hirani, 2009).

When assessing aerobic fitness, especially in trained populations it is clear that, sport-specific testing is advantageous (St Clair Gibson *et al*., 1998). Thus by employing a sport-specific fitness test, a representative value for fitness may be obtained, enabling exercise intensity as a percentage of $\dot{V}O_{2\,peak}$ to be established as accurately as possible. Where appetite and food intake responses to sport-specific exercise are of interest, use of sport-specific fitness tests to define any exercise bout is of clear importance.

Subjective indicators of exercise intensity, such as ratings of perceived exertion, can also be used in conjunction with objective measures of $\dot{V}O_{2\,peak}$ percentage (Borg, 1998), to establish how much effort individuals feel they are contributing during an exercise bout. Ratings of perceived exertion were first introduced by Gunnar Borg, who developed a 6-20 Ratings of Perceived Exertion scale (Borg, 1998) for use with adult populations. However, although typically used with paediatric populations, this scale was suggested to be unsuitable for use with young people as they found the numerical values 6-20 difficult to interpret [for an extensive review please see Eston & Lamb (2000)].

Consequently, child-specific ratings scales have been developed, with the most accepted being the Children's Effort Rating Table (Eston *et al*., 1994) and more recently the Pictorial Children's Effort Rating Table (Eston & Lamb, 2000). Compared to the Borg scale, these scales have fewer possible responses, a range of numbers (1-10) more familiar to young people and descriptors of exercise effort chosen by young people themselves (Eston & Parfitt, 2007). By combining numerical and pictorial ratings of perceived exertion, the Children's Effort Rating Table and the Pictorial Children's Effort Rating Table are believed to be more appropriate with regards to developmental factors for use with young people (Eston & Parfitt, 2007). More specifically, the Pictorial Children's Effort Rating Table portrays a child running up a 45º stepped gradient at five stages of exertion, corresponding to ratings of 2, 4, 6, 8 and 10. With regards to adolescent populations the Pictorial Children's Effort Rating Table has been developed and validated for effort estimation and effort production tasks (Yelling, Lamb & Swaine, 2002). To develop the Pictorial Children's Effort Rating Table, the cited authors recruited 48 adolescent boys and girls (12-15 years) and asked them to complete play and running activities, whilst focussing on sensations of breathlessness, body temperature and muscle aches during the physical activity. Each participant was then asked to identify the position which reflected their effort perception using a copy of the Children's Effort Rating Table and five pictorial descriptors. The format of the Pictorial Children's Effort Rating Table was then developed by recording the frequency with which the participants positioned the pictorial descriptor at points along the scale. Subsequently, the same research group (Yelling, Lamb & Swaine, 2002) explored the validity of the Pictorial Children's Effort Rating Table in a different group of 48 adolescent boys and girls (12-15 years), over two exercise trials. The first trial involved five 3 minute incremental stepping exercise bouts followed by a 2 minute recovery. During the last 15 seconds of each bout heart rate and ratings of perceived exertion, using the Pictorial Children's Effort Rating Table were recorded. An increase in exercise intensity led to a simultaneous increase in ratings of perceived exertion and heart rate. The second trial took place 7-10 days after the first and involved four intermittent 4 minute bouts of steppig exercise, which matched randomly assigned ratings of perceived exertion at 3, 5, 7 and 9 on the Pictorial Children's Effort Rating Table. The participants were asked to regulate how hard they were working (exercise intensity) to match each Pictorial Children's Effort Rating Table value, using verbal feedback from the participants the step height and frequency were altered by the researchers. In the last 15 seconds of each exercise bout heart rate and power output were recorded. Yelling, Lamb and Swaine (2002) found that adolescent boys and girls are able to differentiate between different intensities of exercise, using the Pictorial Children's Effort Rating Table. Consequently, the findings of Yelling, Lamb and Swaine (2002) suggest that adolescent girls should successfully be able to control the intensity that they exercise at, according to ratings from the Pictorial Children's Effort Rating Table.

2.1.4 Summary of Special Considerations

When comparing groups of adolescents in appetite and exercise intervention studies, biological maturity, body composition and fitness status need to be considered to ensure that the data gathered is collected and interpreted in the most accurate way possible. The logistics of the study with regards to the age of the participants and time frame available must be considered in order to allocate the best techniques to establish biological maturity, body composition, exercise intensity and ratings of perceived exertion. Thus from the previous discussion it seems that the prediction of age at peak height velocity should be used to assess biological maturity (Mirwald *et al*., 2000) and the Bod Pod should be used when assessing 2 compartment (fat mass and fat-free mass) body composition in paediatric populations (Fields & Goran, 2000). Tests of aerobic fitness should employ a sport-specific exercise protocol, alongside an appropriate assessment of ratings of perceived exertion using a measure such as the Pictorial Children's Effort Rating Table (Yelling, Lamb & Swaine, 2002).

2.2 Appetite and Energy Intake Regulation

Appetite, per se, can be used as an umbrella term which encompasses a variety of parameters associated with eating behaviour, including hunger, the drive for energy, and selection of specific nutrients and tastes (King, Tremblay & Blundell, 1997).

According to De Castro (1999) several variables influence appetite and energy intake, namely physiology and genetics, internal and external rhythms and psychological and socio-cultural factors. These are thought to be a consequence of past experiences, the present environment and anticipation of future events (De Castro, 1999; Stubbs *et al*., 2000). In consideration of all these factors De Castro (1999) emphasises that it is difficult to comprehend how appetite and energy intake are regulated.

There are an array of peripheral signals which regulate feeding behaviour and thus body mass maintenance. These can be classified as short-term regulators and long-term regulators of appetite (Havel, 2001). In the short-term, Blundell, Goodson and Halford (2001) differentiate between appetite regulation being focussed around hunger and the termination of eating (satiation). With regards to satiation, the gastrointestinal tract, macronutrient intake, circulating factors, the liver and nutrient stores have all been highlighted as peripheral factors regulating appetite and energy intake (Stubbs, 1999). An extensive review of these factors is provided by Stubbs (1999), although a diagrammatic representation is provided in figure 2.1.

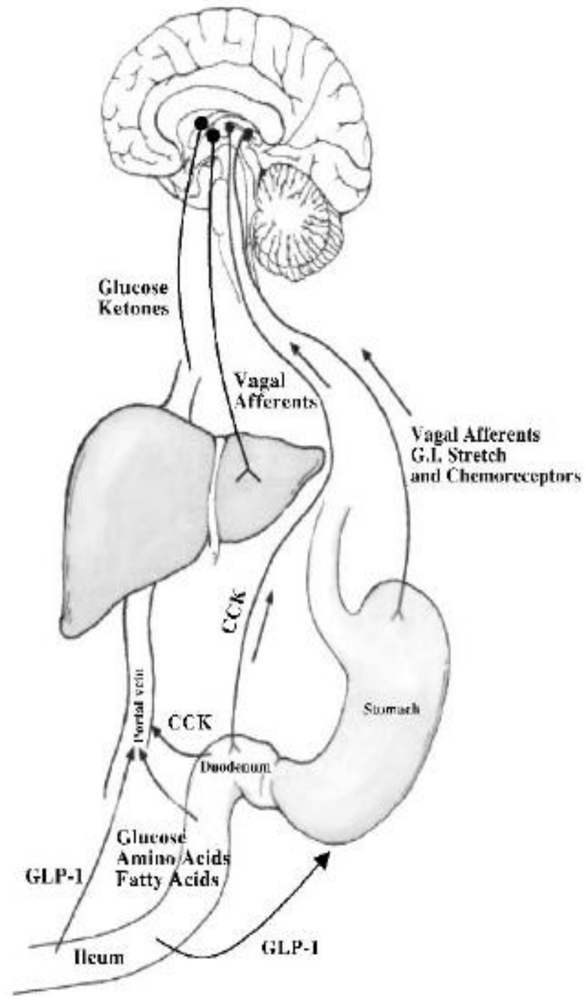


Figure 2.1. Short-term appetite regulation. Taken from Havel (2001, p. 964). Cholecystokinin (CCK), Gastrointestinal (G.I.), Glucagon-like peptide-1 (GLP-1).

2.2.1 Hunger and the Role of Ghrelin

The initiation of eating or stimulation of appetite is known as hunger (King, Tremblay & Blundell, 1997). Individuals tend to use expressions such as ‘being hungry’, to describe a sensation generated by a combination of previous experiences, environmental influences and physiological factors (Stubbs, Ferres & Horgan, 2000).

Exercise is an effective method used to increase energy expenditure, thus potentially influencing hunger. Therefore, if energy expenditure is increased through exercising, one would assume that feelings of hunger would increase and thus energy intake would also increase to compensate for the energy expended during the exercise bout. Interestingly, this does not seem to be the case, with the general consensus from the paediatric literature being that a loose coupling between energy intake and exercise-induced energy expenditure occurs (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). This is extensively reviewed in section 2.5 of this thesis. Lean individuals must however compensate in terms of energy intake in response to elevated exercise-induced energy expenditures at some point in order to maintain a stable body mass (Woods *et al*., 1998; Dodd, 2007). The energy cost of growth (approximately 5 Kcal/g of body mass) (Millward, Garlick & Reeds, 1976) must also be considered, since during the pubertal growth spurt a slight positive energy balance is required where energy intake exceeds energy expenditure by 1-2% (Livingstone *et al*., 1992b; Livingstone, & Robson, 2000). This is especially the case for trained individuals where energy deficits are likely to be induced frequently, although body mass and energy balance are maintained. The maintenance of energy balance introduced the notion of a set-point theory, implying the existence of a set-point which regulates body weight (Paradis, Philippe & Cabanac, 2006). This theory suggests that energy imbalances are accurately restored through negative feedback mechanisms either by increasing or decreasing energy intake (Stubbs, 1999). Unfortunately, if such a set-point exists in humans, it is not a strong enough mechanism, as is evident from the current obesity epidemic in children and adolescents, with the Health Survey for England (2008) concluding that 31% of boys and 29% of girls aged 2-15 years were overweight and 17% of boys and 15% of girls aged 2-15 years old were classed as obese (Craig, Mindell & Hirani, 2009).

In terms of short-term peripheral signals of hunger, sensory inputs are of importance in the regulation of appetite and energy intake (Stubbs, 1999), with signals such as the taste, smell, sound and sight of food contributing to the ingestion of foods, certainly in terms of the amount of food consumed (Friedman, Horn & Ji, 2005). In response to such cues, Cannon and Washburn (1912) proposed that a further primary hunger signal which initiates eating is gastric contraction.

The stomach also releases ghrelin, which is an appetite stimulant that was first identified in 1999. To date there is a dearth of information regarding ghrelin concentrations and fluctuations in children and adolescents, therefore in this section adult data will mainly be discussed. Ghrelin is a 28-amino acid peptide hormone with an acyl side chain which is imperative for its biological action (Kojima *et al*., 1999) and is the only known orexigenic (appetite stimulant) hormone (Wren *et al*., 2001; Cummings, 2006), stimulating neuropeptide Y and agouti-related peptide and therefore hunger (Wren *et al*., 2001). Consequently, ghrelin influences the short-term regulation of energy intake in humans by surging before meals, thus initiating meal consumption (Cummings, 2006). Ghrelin does not alter the size of meals consumed but instead increases the number of meals initiated, therefore elevating energy intake levels (Cummings & Overduin, 2007).

Ghrelin concentrations are then suppressed following the ingestion of nutrients, with carbohydrates being the most effective, then proteins, followed by fats (Cummings & Overduin, 2007). One hour after a meal plasma ghrelin concentrations are known to reach their lowest levels (Cummings *et al*., 2001), with ghrelin levels being proportional to the amount of ingested calories (Callahan *et al*., 2004). Interestingly, in healthy adults, changes in the ghrelin concentration following a meal are smaller compared to after an overnight fast (Cummings *et al*., 2001). Ghrelin is also involved in long term regulation of appetite and thus energy balance (Neary, Goldstone & Bloom, 2004; Tschöp, Castañeda & Pagotto, 2004). Total ghrelin is a combination of acylated ghrelin, which is the most active form of ghrelin, and non-acylated ghrelin (80-90%) (Hosoda *et al*., 2004; Ghigo *et al*., 2005). Non-acylated ghrelin is unable to bind and activate the growth hormone-secretagoue receptor and is therefore devoid of any affects on the endocrine axis (Ghigo *et al*., 2005), whereas acylated ghrelin has the ability to bind and therefore cross the blood-brain barrier (Kojima *et al*., 1999; Murphy & Bloom, 2006). Acylated ghrelin posses pituitaric and pancreatic endocrine activities and consequently is considered to be important for appetite regulation (Broglio *et al*., 2003) and is therefore a more appropriate objective measure of appetite or more specifically hunger.

There are very few studies exploring total ghrelin or more specifically acylated ghrelin concentrations in paediatric populations. The lack of studies in this population group may be due to the high cost and complexity of the acylated ghrelin assay and also logistical reasons such as the difficulties encountered using cannulation procedures in young people. Of the small amount of paediatric ghrelin data available, adolescent girls who regularly take part in systematic training have been shown to have higher plasma ghrelin concentrations compared to their untrained peers (Jürimäe *et al*., 2007a). Jürimäe et al. (2007a) categorised 25 adolescent girls (11-16 years) as trained swimmers and a further 25 adolescent girls (11-16 years) as untrained. The girls were further divided according to maturation status. The trained girls had significantly higher mean plasma ghrelin levels compared to their untrained peers (maturation group 1: 1152.1±312.9 versus 877.7±114.8 $pg{\cdot}mL^{-1}$, respectively; maturation group 2: 1084±252.5 versus 793.4±164.9 $pg{\cdot}mL^{-1}$, respectively) (p<0.05). These results support the suggestions by Ravussin et al. (2001) and Neary, Goldstone and Bloom (2004) that ghrelin levels are responsive to a negative energy balance.

It has also been postulated that ghrelin levels are related to circulating glucose and insulin levels in adults. However research suggests that the nature of this relationship is unclear, with some studies suggesting that insulin and glucose have suppressive affects on ghrelin (Shiya *et al*., 2002; Flannagan *et al*., 2003; Murdolo *et al*., 2003) and others that they do not (Broom *et al*., 2007; Jürimäe *et al*., 2007b; Jürimäe, Jürimäe & Purge, 2007). To date there is a dearth of information regarding this interaction in children and adolescents. The relationship between ghrelin, insulin and glucose is clearly worthy of investigation in young people. In adults, hunger has been linked to circulating glucose levels (Melanson *et al*., 1999), rates of glucose absorption (Kishnamacher & Mickleson, 1987) and insulin activity (Leathwood & Pollet, 1988) and it has also been suggested that ghrelin concentrations and subjective hunger scores are positively correlated (Neary, Goldstone & Bloom, 2004).

2.2.2 Assessing Appetite Using Visual Analogue Scales

In appetite and exercise studies, subjective appetite and energy intake are normally assessed concurrently, to provide an insight into behaviours and mechanisms which influence energy balance (Stubbs, Ferres & Horgan, 2000). A quantitative measure of energy intake can be conducted via self-reported, weighed food diaries and 24-hour recall interviews and these methods are reviewed later in section 2.3. A subjective assessment of the appetite parameters hunger, prospective food consumption and fullness can however be made using several methods and these will now be examined.

There are generally two techniques employed to measure such appetite parameters, namely visual analogue scales (VAS), which are continuous scales and Likert scales, which take the form of fixed-point scales. Typically, VAS take the form of 100-150 mm horizontal lines, with descriptive anchor phrases placed at either end of the line (Wewers & Lowe, 1990). This convenient, easy and quick to administer technique (Wewers & Lowe, 1990) requires the participant to respond to a question by placing a vertical mark between the anchor phrases. To obtain a score from the VAS, the distance from the left hand side of the scale to the individuals mark is measured in millimetres (Wewers & Lowe, 1990). For example, VAS measuring hunger could be labelled *not at all hungry (0)* on one end and *extremely hungry (100)* (Hill & Blundell, 1998). An example of a fixed point scale and a VAS to assess hunger is provided in figure 2.2.

"How hungry do you feel?"

1)

1 2 3 4 5 6 7

Not at all hungry Extremely

hungry

2)

Not at all hungry Extremely

hungry

Figure 2.2 1) a fixed point scale and 2) a visual analogue scale (VAS) example from Hill & Blundell (1998, p. 1016).

Chambers and Craig (1998) suggest that especially young children tend to respond to rating scales in an extreme way. However, it must be noted, that the descriptive anchor phrases should be positioned beyond the ends of the VAS to avoid this issue (Wewers & Lowe, 1990). Through providing careful instructions and familiarisation with VAS, the problems encountered with regards to extreme responses can be eliminated (Price *et al.*, 1983).

The use of VAS to measure hunger, prospective food consumption and fullness these parameters are more appropriate compared to using fixed-point scales. An assumption of fixed-point scales is that, for example, a score of four is twice as much as if an individual rates a subjective parameter as two. There is no evidence to suggest that along the length of the VAS a score of 80 mm represents a score twice as much as 40 mm (Wewers & Lowe, 1990). This seems logical since the result is dependent on the individual's unique interpretation of the anchor phrases and also on their current knowledge and experience (Wewers & Lowe, 1990). The numerical values derived from VAS therefore have properties of a ratio scale rather than an interval scale (Price *et al.*, 1983).

Visual analogue scales have been used in three published studies for assessing appetite parameters following exercise in 9-10 year old girls (Moore *et al*., 2004), 12-14 year old girls (Rumbold & Dodd, 2007) and also in 12.5±0.4 year old boys (Bellissimo *et al*., 2007). The ability of children and adolescents to use VAS has been extensively demonstrated when assessing other psychological domains such as pain (Shields *et al*., 2003). Shields et al. (2003) suggest that it is difficult to assess a child's understanding of how to use a VAS, based on their responses. Visual analogue scales require individuals to extrapolate a subjective sensory experience into a linear format (Gift, 1989), leading Shields et al. (2003) to suggest the notion that cognitive ability could better predict children's ability to use VAS instead of age. This group presented 40 kindergarten children (5.8±0.4 years old) with different size circles, which they had to rate, by size, on VAS. Only 42% of the children could accurately use the VAS, suggesting that age combined with estimated intelligence quotient was the best predictor of a child's ability to use a VAS (88% accurate). Although such research has not been conducted in terms of appetite sensation, it does suggest that older children (9-12 years old) are better able to translate their subjective experiences onto a VAS, which may be due to advanced brain development and enhanced cognitive abilities. Thus they are therefore able to respond in a more graded manner, between the anchor phrases on a VAS (Chambers & Craig, 1998).

2.2.2.1 Cognition and Brain Development through Adolescence

With regards to the study of young people's cognition, Piaget identified four major periods of cognitive development, namely sensorimotor (birth to 2 years), preoperational (2-7 years), concrete operations (7-11 years) and formal operations (11 years and beyond). According to Piaget's Cognitive-Development Theory, young people enter the formal operational stage around the age of 11 years old, whereby the ability to think scientifically and abstractly is developed (Berk, 2006). Indeed, one characteristic of the formal operational stage is propositional thought, where it is believed adolescents can evaluate the logic of verbal statements better than younger children (Berk, 2006). Other characteristics of this stage include improvements in reasoning, information processing, expertise, and multidimensional, planned and hypothetical thinking during the transition between late childhood and middle adolescence (Steinberg, 2005). Overall at the core of cognitive development in adolescent populations are a more conscious, self-directed and regulated mind set (Steinberg, 2005). In addition the link between cognitive development and its social context has also been explored, more specifically with regards to the development of judgment, decision making and risk taking (Steinberg & Cauffman, 1996). More recently, Keating (2004) emphasised that adolescents function by interacting cognitive processes with social and emotional factors which then impact on adolescent thinking in reality.

Alongside such cognitive development, the growth of the brain must also be considered, although the link between brain maturation and cognitive development during adolescence is tenuous (Steinberg, 2005). According to Steinberg (2205) the prefrontal cortex of the brain contains large numbers of neurons and synapses, making it an extremely complex structure. During adolescence, myelination of the frontal lobes occurs, which further supports the development of complex cognitive abilities (Sowell *et al*., 2002) and increases the efficiency of information processing (Paus, 1999). Indeed, Paus (2005) suggests that throughout adolescence maturational brain processes continue. The impact of this continued development suggests that during the second decade of life changes in brain structure and function occur in regions of the brain associated with response inhibition, risk, reward and emotional regulation (Steinberg, 2005). To understand psychological development throughout the adolescent period two aspects of brain development must be considered (Steinberg, 2005). In areas of the brain such as the regulation of behaviour, emotion, perception and the evaluation of risk and reward, the majority of development in these areas occurs during adolescence. Secondly, changes in arousal and motivation due to puberty affect regulatory competence in these areas during adolescence. Although during late adolescence maturation of the frontal lobes helps facilitate regulatory competence (Steinberg, 2005). Therefore taken together these factors suggest that adolescents can express their previous experience better, which may help to explain why adolescent populations can complete VAS more effectively compared to younger children (Foster *et al*., 2008b).

2.2.3 Summary of Appetite and Energy Intake Regulation

From the available literature it appears that VAS have been used for assessing subjective appetite in appetite and exercise research studies involving paediatric groups (Moore *et al.*, 2004; Gately *et al*., 2007; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). It has also been suggested that due to enhanced cognitive abilities (evaluate verbal statements, improvements in reasoning, information processing), the use of extreme anchors and habituation with the VAS, older children (9-12 years) are able to translate their subjective experiences onto a VAS more successfully than younger children. This therefore enables subjective appetite parameters such as hunger, prospective food consumption and fullness, to be assessed in conjuction with energy intake, in appetite and exercise research studies involving adolescent populations. However, a more objective measure of appetite such as the sampling of the appetite hormone ghrelin would be useful to consolidate the subjective appetite data and findings, in any trial studying appetite regulation in paediatric populations.

2.3 Assessment of Energy Intake

Assessing energy intake in adolescent girls following exercise-induced energy expenditure, in addition to a measure of 24-hour energy expenditure, enables 24-hour energy balance to be quantified. This provides insights into the calorific needs of trained young people following exercise and also information pertaining to when energy balance is restored in this population. In order to obtain a representative value of 24-hour energy balance, 24-hour energy expenditure and 24-hour energy intake must be assessed as accurately as possible. Intervention studies, which have examined energy intake responses to exercise-induced energy expenditure in young people in a controlled setting, have employed observation whilst food was provided *ad libitum* (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008) to provide a value for energy intake. This has also been the technique of choice with regards to trained adolescent populations (Rumbold & Dodd, 2007). Techniques which are most commonly used in young people (Dodd, 2007) and where a measure of habitual energy intake is required during a free-living period over several days, are food frequency questionnaires, self-reported estimated or weighed food records and 24-hour recall interviews. Unlike the observational technique which has been employed in all of the paediatric exercise and appetite studies to date (Moore *et al*., 2004; Rumbold & Dodd, 2007; Bellissimo *et al*., 2007; Dodd, Welsman & Armstrong, 2008), food frequency questionnaires, self-reported estimated or weighed food records and 24-hour recall interviews enable free-living energy intake to be assessed over several consecutive days. In light of this, the following section will review the validity of self-reported weighed food records and 24-hour recall interviews, for assessing 24-hour free-living energy intake in normal-weight girls (children and adolescents).

2.3.1 Self-Reported, Weighed Food Diaries

Food records are regarded as the ‘gold standard’ technique used to assess dietary intake (Black *et al*., 1991; Ashley & Bovee, 2003), in both adults and young people. Typically self-report food diaries are collected for 3, 4, 5 or 7 consecutive days (Rutishauser & Black, 2002). According to Black et al. (1991) 7 day self-reported, weighed food diaries offer the best compromise between precision, researcher burden and participant compliance. The latter is especially important in young people where motivation and compliance issues must be considered when choosing an energy intake assessment technique (Livingstone & Robson, 2000). When completed properly, self-report weighed food records provide a measure of actual food intake, normally at the time of food consumption (McPherson *et al*., 2000) and are therefore known as prospective techniques

(Ashley & Bovee, 2003). Self-report food records are appropriate for collecting 24-hour free-living energy intake data, as it is the responsibility of the individual to weigh and record all food and drink items that they consumed during a 24-hour period (Hill, Rogers & Blundell, 1995). This is known as the weighed inventory method, where food and beverages are weighed in the form they are consumed along with any left-over items (Rutishauser & Black, 2002). Rutishauser and Black (2002) suggest that to increase the accuracy of self-reported weighed food records, dietary scales need to be robust and able to weigh up to a mass of 2 kg with a precision of ± 2 g. The amount of food consumed can also be measured in volume, where participants record the quantity of food items eaten in terms of household measures (Hill, Rogers & Blundell, 1995). In addition to the amount of food consumed, the participants are asked to record information such as brand names, recipes of food dishes and preparation and cooking methods, in order to facilitate the subsequent dietary analysis (McPherson *et al*., 2000). Consequently, self-reported weighed food records require motivated and committed participants (Johnson, 2002).

The validity of self-reported weighed food records for assessing free-living energy intake in young people has been previously explored (Livingstone *et al*., 1992b). Livingstone et al. (1992b) recruited 29 non-obese girls aged 7-18 years old and grouped them in terms of their age (7, 9, 12, 15 and 18 years). Over seven consecutive days energy intake and daily energy expenditure were simultaneously assessed. The doubly labelled water technique was employed to assess daily energy expenditure whilst self-reported weighed food records were used to quantify daily energy intake. The parents of the 7 and 9 year old children were asked to record the food and fluid intake of their child, whilst the 12, 15 and 18 year olds were told to take responsibility for this themselves with support from their parents. The participants were provided with the necessary items for recording a food diary, such as dietary scales and a logbook, which provided written instructions and examples of how to fill out the food record. Prior to the free-living energy intake data collection all participants and parents were provided with a demonstration of the weighing process and were asked to complete the process in order for the research team to assess their competence. Livingstone et al. (1992b) emphasised that the children and adolescents should not attempt to estimate food portions away from home as this may have interfered with eating habits. Using paired t-tests Livingstone et al. (1992b) demonstrated that there was no significant difference between estimates of daily energy expenditure and energy intake assessed using self-reported, weighed food records for the 7 and 9 year old girls. The mean (±SD) energy intake expressed as a percentage of energy expenditure, to provide an indication of reporting accuracy, yielded highly favourable results for the 7 and 9 year old age groups, 91.7±13.8 and 101.2±18.8%, respectively. For the adolescent girls (12, 15 and 18 years old) there was a trend for underreporting of energy intake with increasing age (especially between the ages of 12 and 15 years old), 84.7±12.4, 68.0±20.5 and 77.4±20.2%, respectively. A trend for under-reporting of energy intake was also identified for the normal-weight adolescent girls with increasing body mass index (17.6 kg/m^2, 19.7 kg/m^2 and 26.0 kg/m^2), 83±13, 86±10 and 62±21%, respectively. Livingstone et al. (1992b) concluded that self-reported, weighed food records provide a valid estimate of free-living energy intake, on a group level. For girls aged 7-9 years old, however there seems to be a systematic negative bias in free-living energy intake reporting by adolescent girls. The cited authors suggest that such favourable results for the 7-9 year old girls is a consequence of parental or adult control over the recording process, since the younger girls had very little or no input, whereas the adolescent girls (12-18 years old) were responsible for recording their own food and fluid intake for the seven consecutive days.

Similarly to Livingstone et al. (1992b), Bratteby et al. (1998) suggests that adolescent girls under-report free-living energy intake. Fifty adolescent girls (15.0±0.1 years old) participated in the study and using the same methodology of Livingstone et al. (1992b) energy intake was assessed using self-reported, weighed food records over seven consecutive days, whilst energy expenditure using the doubly labelled water technique was simultaneously assessed. Bratteby et al. (1998) told the girls not to estimate food portions outside of the home. When mean (±SD) energy intake was expressed as a percentage of energy expenditure, this equated to 78.3±16.4%. Bratteby et al. (1998) concluded that this result was comparable to that identified by Livingstone et al. (1992b) in adolescent girls of a similar age (15 years old), where self-reported daily energy intake accounted for 68.0±20.5% of daily energy expenditure

In the first longitudinal study of this nature, Bandini et al. (2003) reiterated the findings and conclusions of Livingstone et al. (1992b) and Bratteby et al. (1998) with regards to adolescent girls. Bandini et al. (2003) assessed daily energy intake using self-reported weighed food records and daily energy expenditure using doubly labelled water, simultaneously for seven consecutive days, of the same 21 girls aged 10, 12 and 15 years old. At age 10 years old all of the girls were classified as normal weight, however through age 12 and 15 years several girls were identified as overweight. The results demonstrated that with increasing age and increasing body mass index (BMI) the girls under-reported their daily energy intake. At age 10, 12 and 15 years, daily energy intake, adjusted for changes in body mass, accounted for 82±17, 71±24 and 66±20% of daily energy expenditure, respectively. Between the ages 10 and 12 years there was a significant difference in energy intake reporting accuracy (*p*=0.03), which was also the case between the ages 10 and 15 years (*p*=0.001). There was also deterioration in energy intake reporting accuracy between the ages of 12 and 15 years, however this was not significant. Bandini et al. (2003) concluded that the favourable agreement between daily energy intake and daily energy expenditure at age 10 years was due to the increased parental involvement in the energy intake recording process, in comparison to at age 12 and 15 years.

Instead of using self-reported, weighed food records, Bandini et al. (1990) investigated the validity of self-reported estimated food records, identifying a very similar level of negative bias to those previously identified (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003). Over 14 consecutive days in 28 non-obese adolescent boys and girls (14.7±2.0 years old), energy expenditure data was derived from doubly labelled water whilst simultaneously the participants estimated their energy intake using household measures. Mean (±SD) daily energy intake, after adjustments for changes in body composition, account for 80.2±22.6% of daily energy expenditure, demonstrating an under-reporting of energy intake. The findings of this study suggest that under-reporting of energy intake in adolescent populations is unrelated to the dietary assessment technique used, since the use of weighed and estimated food records to quantify energy intake yield similar amounts of bias (Livingstone & Robson, 2000).

Taken together these results suggest that 15 year old adolescent girls are likely to under-report their energy intake by approximately 20% (Bandini *et al*., 1990; Bratteby *et al*., 1998) to 30% (Livingstone *et al*., 1992b; Bandini *et al*., 2003) when self-reported weighed or estimated food records are utilised. There is also a trend for normal weight adolescent girls of increasing age (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003) and increasing body mass index (Livingstone *et al*., 1992b; Bratteby *et al*., 1998) to underreport their energy intake using self-reported weighed food records.

2.3.2 24-hour Recall Interviews

The 24-hour recall technique requires participants to estimate portion sizes (Rutishauser & Black, 2002; Ashley & Bovee 2003). Unlike the self-reported weighed food record technique, which is a prospective method of dietary assessment, the 24-hour recall interview is retrospective (Ashley & Bovee, 2003) whereby information on previous food intake is collected (Hill, Rogers & Blundell, 1995). Typically, 24-hour recall interviews involve trained individuals conducting a short interview (Johnson, 2002) to obtain information regarding the previous 24-hour food consumption (Hill, Rogers & Blundell, 1995). A two pass method (Ashley & Bovee, 2003) or a multiple pass 24-hour recall

(Rutishauser & Black, 2002) is normally used, where the participants are prompted for information using two or three phases, respectively. In the two-pass method, overall eating events are reviewed, followed by prompts to ascertain portion sizes, additional condiments, brand names, extra items and preparation and cooking methods (Ashley & Bovee, 2003). The multiple pass method is similar, however it includes a third phase where the list of foods is reviewed in order to prompt for additional food items or eating episodes (Rutishauser & Black, 2002).

An advantage of 24-hour recall interviews is that they have a low participant burden (Hill, Rogers & Blundell, 1995) and are cheap to administer (Livingstone & Robson, 2000). Additional benefits include that they are quick to conduct (Livingstone & Robson, 2000) and when administered unannounced modification of an individual's dietary habits are unlikely (Johnson, 2002). These benefits mean the 24-hour recall interview technique is well suited to use with children and adolescents and indeed ideal for use in free-living environments (Samuelson, 1970). It must however be recognised that the information collected during a 24-hour recall interview is largely dependent on the accuracy of the adolescents' verbal episodic memory (a type of declarative memory, which requires conscious recall of contextual information) (Smith & Foster, 2008). Perception (interpretation of the incoming stimuli) also play a major role in children's and adolescent's ability to perform well on a 24-hour recall interview (Baranowski & Domel, 1994; Foster *et al*., 2008b). For adolescent populations who are seen to have fully matured cognitive function, memory and perception issues may not be obstacles (Livingstone & Robson, 2000). Indeed, with regards to perception and memory, Foster et al. (2008b) investigated the length of time required between food consumption and a recall interview, for portion size to be estimated as precisely and accurately as possible. One hundred and eight young people participated in the study and were divided into three groups depending on their age: 4-6 years, 7-10 years and 11-16 years. The children and adolescents were asked to estimate portion size when the food was in front of them, just after eating and 24-hours after food consumption. The accuracy of the recall was defined as how close the children's estimates of portion size were to the actual weight of the food served, whereas precision was defined as the variability of the individual estimates (Foster *et al*., 2008b). Foster et al. (2008b) concluded that on a group level, a dietary recall performed 24-hours after food consumption was not significantly different to a recall performed with food in front and just after eating in terms of portion size estimation, thus 24-hours is an appropriate length of time to conduct a recall interview. Interestingly, Foster et al. (2008b) also identified that regardless of the timing of the dietary recall, the precision and accuracy of portion size estimation increased with age, thus younger children (4-10 years old) tended to overestimate portion sizes and were therefore less accurate and less precise compared to older children and adolescents (11-14 years old). A review of the ability of children to report their food intake with regards to cognition is provided by Baranowski and Domel (1994).

Indeed, food per se has been shown to influence cognition during adolescence (Smith & Foster, 2008). More specifically, Smith and Foster (2008) explored verbal episodic memory (a type of declarative memory, which requires conscious recall of contextual information) in 32 healthy adolescents (15.6±0.9 years) after oral glucose administration. In comparison to a placebo drink, the ingestion of a glucose drink elicited enhanced verbal episodic memory performance in the adolescent population sampled. This suggests that the types of foods consumed prior to completing a 24-hour recall interview may be influential in how well foods are recalled.

Twenty four hour recall interviews have successfully been used to assess energy intake in adolescent populations (Frank *et al*., 1977; Nicklas *et al*., 1991) and also in young female athletes involved in team sports (Perron & Endres, 1985). Perron and Endres (1985) assessed the energy intake of 26 female volleyball players (13-17 years old) using 24-hour recall interviews, and found mean daily energy intake equated to 7.56 $MJ \cdot d^{-1}$. However, energy expenditure was not determined, therefore Perron and Endres (1985) were unable to identify if energy intake was sufficient enough to meet the energy requirements of the female volleyball players (Thompson, 1998). Having not determined energy expenditure this made it difficult for Perron and Endres (1985) to determine how accurately energy intake was reported. Energy intake was however identified to be underreported when compared to doubly labelled water values derived from non-athletic groups of a corresponding age (Thompson, 1998).

No study as yet has employed doubly labelled water as a reference technique to investigate the validity of 24-hour recall interviews for assessing energy intake in young athletes and more specifically adolescent girls who play team sports. Two studies have however explored the validity of 24-hour recall interviews in young children (4-7 years old) (Johnson, Driscoll & Goran, 1996) and older children (9.5±1.4 years old) (Lindquist, Cummings & Goran, 2000), using doubly labelled water as a reference technique. In addition, these studies are two of the few available validation studies, which have explored a 24-hour period and not only a portion of the day or individual meals and eating occasions (McPherson *et al*., 2000).

Lindquist, Cummings and Goran (2000) recruited 30 children (boys and girls) aged 9.5±1.4 years. Energy expenditure was quantified using the doubly labelled water technique and energy intake was assessed using three 24-hour recall interviews, over two week days and one weekend day. When mean energy intake was compared to mean energy expenditure there was a slight difference of 0.04 $MJ \cdot d^{-1}$, illustrating excellent agreement on a group level. However, the correlation between the 24-hour recall interviews and energy expenditure was weak ($r=0.32$; $p=0.08$). These findings led the authors to conclude that on a group level 24-hour recall interviews are a valid technique to use, however the validity is questionable on an individual basis (Lindquist, Cummings & Goran, 2000).

Energy intake assessment techniques must be considered with specific regard for the age of the children or adolescents that they are likely to be used in. This is especially important when attempting to use techniques to assess energy intake in adolescent populations, which have only been validated in younger children. Due to the increased likelihood of under-reporting with increasing age in normal weight adolescent girls described previously (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003), it is likely that results from studies using young children cannot be extrapolated to adolescent populations.

With regards to adolescent girls (12.5–14.5 years old) Greger and Etnyre (1978), compared actual measured energy intake against information collected from 24-hour recall interviews. Seventeen girls were involved in a 30 day metabolic study, on days two and five of the study, 17 and 15 girls, respectively, completed a 24-hour recall interview. When mean (±SD) recalled energy intake was compared to mean (±SD) actual, pre-weighed energy intake for day two and five, there were no significant differences, 8.40±1.71 versus 9.12 $MJ \cdot d^{-1}$ and 10.20±2.38 versus 10.39 $MJ \cdot d^{-1}$, respectively. Greger and Etnyre (1978) therefore concluded that 24-hour recall interviews are a valid method for assessing energy intake in groups of adolescent girls and when performed more than once accuracy is improved.

Taken together these results suggest that on a group level 24-hour recall interviews provide a valid estimate of energy intake in older children (9.5±1.4 years old) (Lindquist, Cummings & Goran, 2000) and adolescent girls (12.5–14.5 years old) (Greger & Etnyre, 1978). It is likely such validity is somewhat dependent on the ability of older children and adolescents to estimate portion sizes more accurately than younger children (Foster *et al*., 2008b) due to their enhanced cognitive abilities (Livingstone & Robson, 2000) in terms of perception, conceptualisation and memory (Foster *et al*., 2008b).

2.3.3 Combination of Energy Intake Assessment Techniques

Livingstone and Robson (2000) suggest that estimates of energy intake are enhanced when two methods of dietary assessment are used in conjunction with one another. Studies that have combined energy intake measurement techniques have typically employed one retrospective method and one prospective method (Trabulshi & Schoeller, 2001).

Lytle et al. (1993) investigated the use of 24-hour recall interviews combined with a non-quantified food record, which was used as a memory prompt during the 24-hour recall interview, in 49 children (8 years old). During the 24-hour recall the children were required to estimate portion size, using three-dimensional food models, measuring utensils and tableware. The reference standard was observation by parents and staff, therefore observed energy intake was compared to recalled energy intake. Lytle et al. (1993) found that the children significantly overestimated their energy intake in comparison to observed energy intake, (7.65 ± 2.65 and 6.93 ± 2.33 $MJ \cdot d^{-1}$), respectively. It is however acknowledged that older children and adolescents are more accurate and more precise (11-14 years old) at estimating portion size, with a tendency for overestimating portion sizes during a 24-hour recall interview (Foster *et al*., 2008b), in comparison to young children (4-10 years).

These findings however do demonstrate the potential use of self-reported weighed food records combined with 24-hour recall interviews to assess energy intake in children and adolescents. With regards to young children, this approach eliminates the problems associated with portion size estimation in this population, since the quantity of food consumed can be derived from the food record, whilst specific information regarding drinks, snacks, condiments and additional details about food and drink items consumed can be obtained during the 24-hour recall interview. With regards to normal-weight adolescent girls, who with increasing age, tend to underreport energy intake using self-reported weighed food records (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003), the quantities and types of foods consumed can be obtained from the food record, whilst information from the 24-hour recall interview can be used to substantiate the contents of the food record in an attempt to minimise mis-reporting.

2.3.4 Reasons for Under-reporting of Energy Intake

The research thus far indicates that under-reporting of energy intake is highly likely in adolescent populations (Livingstone & Robson, 2000). It is recognised that reporting bias is not consistent across age-groups (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003) and indeed BMI has an influence (Livingstone *et al*., 1992b; Bratteby *et al*., 1998). The choice of dietary assessment technique is also influential (Hill, Rogers &

Blundell, 1995), with comparable underreporting bias derived from self-reported weighed and self-reported estimated food records (Livingstone & Robson, 2000). However, good agreement on a group level occurs between 24-hour recall interviews and doubly labelled water in older children (Lindquist, Cummins & Goran, 2000), and specifically in adolescent girls using actual measured energy intake as a reference technique (Greger & Etnyre, 1978). With specific regards to adolescent girls, several reasons have been proposed in an attempt to elucidate why this specific population under-reports daily energy intake. Reasons for under-reporting relating to self-reported, weighed food records are described below, since in adolescent girls this seems to be the dietary assessment technique associated with the most bias.

Unlike in younger children where parents take responsibility for recording their child's energy intake using self-reported, weighed food records, adolescents take full responsibility for recording their own energy intake (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Livingstone & Robson, 2000; Bandini *et al*., 2003). Consequently, Bratteby et al. (1998) reported that adolescent girls found recording their own food intake inconvenient and therefore a burden, with adolescent girls also being less interested in participating in this process compared to children (Livingstone & Robson, 2000). In addition, adolescent populations have elevated energy requirements, in combination with unstructured eating behaviours (Livingstone *et al*., 1992b; Livingstone & Robson, 2000) and a significant proportion of eating out of home (Livingstone *et al*., 1992b; Lin, Guthrie & Blaylock, 1996; Livingstone & Robson, 2000). Such factors have been suggested to affect the accuracy of dietary reporting due to forgetfulness and a lack of compliance, caused by the irritation and tedium of recording food items on an hour-to-hour basis (Livingstone & Robson, 2000). In relation to self-reported weighed food records when portion sizes for food and drink items consumed outside the home have not been provided, Livingstone et al. (1992b) suggest that missing portion sizes are substituted with food portions measured within the home. This ensures that free-living eating patterns are not influenced (Livingstone *et al*., 1992b) and also that an additional source of bias is not introduced (Bandini *et al*., 2003). Consequently, self-reported, weighed food records require motivated and compliant adolescent girls (Livingstone *et al*., 1992b; Livingstone & Robson, 2000; Foster *et al*., 2008b). It may be the case that habitually active adolescent girls are more committed than their inactive counterparts, when it comes to reporting energy intake. Being inherently motivated as athletes often are (Gould, 1982), this may improve the quality and accuracy of self-reported energy intake data derived from this special population.

An issue which reduces the recording accuracy of energy intake data, in normal-weight adolescent girls, is dissatisfaction with body weight (Wardle & Beales, 1986), body image and body shape (Livingstone & Robson, 2000). Dietary restraint is also likely to affect individuals who have a high body mass (Herman & Polivy, 1980). Wardle et al. (1992) identified that girls of varying ages (11.8-18.0 years old) who were identified as restrained eaters had significantly higher BMI (p<0.001) compared to children and adolescents of a similar age who were classified as unrestrained eaters. Interestingly, the cited authors also found that perceived body size was a major determinant of restraint in comparison to actual body size (p<0.001). Thus, even in below normal-weight and normal-weight adolescents (12 years old), dietary restraint has been identified (Wardle & Beales, 1986). Edwards et al. (1993) also suggests that college-aged athletes who are conscious of their weight and physique or dissatisfied with their body image are also at a high risk of under-reporting of food intake. However, such issues identified above may not necessarily apply to trained adolescent girls. Such preoccupations with body weight, image and shape typically lead to adolescent girls consciously restricting their food intake to control their body weight, and they are therefore identified as restrained eaters (Herman & Polivy, 1980). Unrestrained eaters are therefore identified as individuals who do not monitor their food intake (Gorman & Allison, 1995). Although dietary restraint is acknowledged in research studies it is not always measured.

2.3.4.1 Measuring Dietary Restraint

There are two questionnaires available to assess dietary restraint in children and adolescents. Firstly, there is the Dutch Eating Behaviour Questionnaire (Van Strein *et al*., 1986a) which contains a 10-item Restraint Scale as part of the 33-item questionnaire, with the remaining items on the Dutch Eating Behaviour Questionnaire consisting of an emotionally cued eating scale and an external eating scale. Secondly, a children's version of the Dutch Eating Behaviour Questionnaire has also been developed, known as the Dutch Eating Behaviour Questionnaire-Children, which includes a 7-item restraint scale for assessing restrained eating in young people (7-12 years old) (Van Strein & Oosterveld, 2008). The original Dutch Eating Behaviour Questionnaire Restraint Scale was intended for use with adolescent populations (Van Strein & Oosterveld, 2008), since during the construction of the Dutch Eating Behaviour Questionnaire restraint scale, the items were sampled in a group of 724 high school girls (15.6±1.5 years old).

Wardle et al. (1992) investigated the predictive validity of the Dutch Eating Behaviour Questionnaire restraint scale. The Dutch Eating Behaviour Questionnaire was administered to 439 girls (11.8-18.0 years old) and 407 boys (11.8-18.0 years old) and a measure of energy intake was derived from 24-hour recall interviews, which was subsequently used as the criterion measure. Wardle et al. (1992) identified that individuals with a high dietary restraint score had significantly lower energy intakes, significantly lower carbohydrate intakes and lower fat intakes ($p<0.001$). These results demonstrated that the Dutch Eating Behaviour Questionnaire restraint scale was able to identify lower energy intake in children and adolescents who were categorised as restrained eaters using the Dutch Eating Behaviour Questionnaire restraint scale. However it has been suggested that a measure of energy intake does not necessarily identify the degree to which an individual eats less than desired, which according to Van Strein et al. (1986b) is the ultimate criterion used to identify restrained eating. Consequently, Van Strein et al. (1986b) indirectly assessed the degree of dietary restraint, by determining the difference between estimated energy requirment (body mass and physical activity level) and energy intake assessed during the study. This provided an estimation of the difference between actual energy intake and desired energy intake and thus dietary restraint. Estimates of energy, fat and sugar intake were obtained using 24-hour recall interviews, over 3 month intervals, in 110 females (31-34 years old). The Dutch Eating Behaviour Questionnaire restraint scale was administered during the second 24-hour recall interview. Van Strein et al. (1986b) identified that the mean energy intake was less than the calculated mean energy requirement (-1.17 ± 2.69 $MJ \cdot d^{-1}$). The relationship between the deviation of energy intake from energy requirements and the level of dietary restraint was identified to have a significant negative correlation ($r=-0.37$; $p<0.01$), thus the higher the level of dietary restraint the lower energy intake consumption. This was also the case for fat ($r=-0.28$; $p<0.01$) and sugar ($r=-0.38$; $p<0.01$) intake. Van Strein et al. (1986b) concluded that the Dutch Eating Behaviour Questionnaire restraint scale possess moderate to good predictive validity.

Concurrent validity, a form of criterion validity, refers to how accurately measurement tools are at identifying an individual's current state with regards to the criterion (Colman, 2001). Thus in terms of restrained eating a questionnaire would have concurrent validity if it measured the current levels of dietary restraint experienced by the participant. It has been identified that individuals classified as restrained eaters can be broken down into two subpopulations, successful and failed dieters (Van Strein, 1997a). Van Strein (1997b) investigated the concurrent validity of the Dutch Eating Behaviour Questionnaire restraint scale, by exploring whether the scale could differentiate between or identify successful and failed dieters. Two-hundred and two adolescent girls (16.5±1.4

years old) were split into two groups (successful and failed dieters), using responses from the Dutch Eating Behaviour Questionnaire and the Revised Eating Disorder Inventory (Garner, 1990). Van Strein (1997b) therefore investigated whether these identifiers corresponded with the classification of the successful and failed dieters as grouped using the responses from the Dutch Eating Behaviour Questionnaire and the Revised Eating Disorder Inventory (Garner, 1990). Indeed this was the case, leading Van Strein (1997b) to conclude that the method of classification had high concurrent validity.

2.3.5 Summary of Energy Intake Assessment

When energy intake is being assessed in exercise and appetite studies, Livingstone and Robson (2000) suggest that it is important to identify adolescent girls who are likely to under-report as a result of dietary restraint. This is also of importance in individuals who train for specific sports. A measure of dietary restraint helps to eradicate a potential source of bias, leading to better study designs and the correct interpretation of energy intake data in these special populations (Livingstone & Robson, 2000). If one is concerned with the internal integrity of a scale which assesses dietary restraint, it seems the Dutch Eating Behaviour Questionnaire restraint scale is superior and the most favourable (Allison, Kalinsky & Gorman, 1992) and is also more favourable in paediatric research due to its recommended use with adolescent populations (Van Strein & Oosterveld, 2008).

Appropriate techniques such as those mentioned, especially in the form of a combined self-reported, weighed food diary and 24-hour recall interview, need to be chosen to assess energy intake in young people involved in free-living exercise and appetite studies. Ideally, where there is the potential for under-reporting of energy intake in adolescent female populations, an agreement study to explore the use of a combined energy intake assessment technique, comparing this method with direct observation would be warranted. By addressing the logisitics of the research, such as the age of the participants along with the study time frame, appropriate energy intake assessment methods can be decided upon. The chosen, justifiable methods can then be utilised in exercise and appetite studies to explore energy intake compensation for exercise-induced energy expenditure. Thus in addition to a measure of energy intake in exercise and appetite studies, it is also pertinent, where possible to obtain an accurate measure of energy expenditure in order to explore energy balance.

2.4 Assessment of Energy Expenditure

Energy expenditure is defined as the energy cost of behaviour, and more specifically the energy required by skeletal muscle to carry out contractions and therefore movement (Lamonte & Ainsworth, 2001). Goran and Astrup (2002) suggest that energy expenditure can be categorised as being the result of three main functions. The function which requires the largest proportion of total daily energy expenditure is basal metabolic rate, accounting for two thirds or 50-70% of total daily energy expenditure (Wong *et al.*, 1996). Basal metabolic rate refers to the energy expended by the body to maintain basic physiological processes, essentially the minimum amount of energy required to sustain life in an awake state (Goran & Astrup, 2002). The thermic effect of food ingestion, known as diet-induced thermogenesis, which encompasses digestion, metabolism and conversion and storage of ingested macronutrients, contributes to approximately 10% of total daily energy expenditure (McArdle, Katch & Katch, 1991). In addition to basal metabolic rate and diet-induced thermogenesis, the energy required by skeletal muscle to perform general physical movement and exercise also contributes to total daily energy expenditure (20-40%) (Blundell, Goodson & Halford, 2001). In order to assess 24-hour energy expenditure, and more specifically 24-hour energy balance, it is most often the case that basal metabolic rate and daily energy expenditure are measured and the data collated to obtain an overall 24-hour value (Livingstone, Robson & Totton, 2000). The energy cost of growth (approximately 5 kcal/g of body mass) (Millward, Garlick & Reeds, 1976) must also be considered, since during the pubertal growth spurt a slight positive energy balance is required where energy intake exceeds energy expenditure by 1-2% (Livingstone *et al*., 1992b; Livingstone, & Robson, 2000).

Indeed, in exercise and appetite studies, energy expenditure and energy intake data have been collated to provide an indication of 24-hour energy balance (Stubbs *et al*., 2004), however this has only been apparent in adult populations, with no information to data regarding 24-hour energy balance in young people. Thus specifically with regards to young lean habitual exercisers, 24-hour energy balance data has the potential to provide insights into when or if individuals ‘compensate’ for exercise-induced energy expenditure in terms of energy intake. Thus to obtain a representative value of 24-hour energy balance, 24-hour energy expenditure as well as 24-hour energy intake must be assessed as accurately as possible. In light of this, the following section will review several methods available to assess daily energy expenditure, resting metabolic rate and thus 24-hour energy expenditure in young people.

2.4.1 Energy Expenditure Measurement Techniques

The choice of energy expenditure measurement technique depends primarily on practicality and financial cost (Dodd, 2007) and the likelihood of compliance in young people (Livingstone *et al.*, 1992a).

Direct calorimetry is regarded as the most accurate technique for assessing energy expenditure in humans (Dauncey & James, 1979). It works on the premise that heat produced by the body, at rest and during exercise, is proportional to energy expended (Lamonte, Ainsworth & Tudor-Locke, 2003). According to Murgatroyd, Shetty and Prentice (1993) this technique has several advantages, providing the most accurate and precise direct measure of energy expenditure, which is ideal as a reference method when validating other energy expenditure techniques. Regardless of these advantages, direct calorimetry is not frequently used (Goran & Astrup, 2002). Murgatroyd, Shetty and Prentice (1993) outline some of the disadvantages associated with direct calorimetry, which in terms of assessing 24-hour energy expenditure are likely to be a hindrance. Initially, the procedure is expensive and requires complex mechanical work by trained and knowledgeable technicians. Since it confines participants to a whole body chamber, known as a whole body calorimeter, behaviour changes are induced, thus limiting its use in assessing habitual or free-living energy expenditure. In addition, since heat loss lags behind that of heat produced through metabolic processes due to heat storage within the body, this means that measurements must start and end in the same thermal state or last long enough so that discrepancies in heat storage are small enough in proportion to the total heat production. Additionally, the resultant information provides no indication of substrates oxidised and their contribution to expenditure of energy and all heat sources must be accounted for, more specifically in the form of food or drink and electrical appliances, which can be arduous. In terms of exercise-induced energy expenditure only information regarding the total amount or volume of exercise performed is provided, with no indication of intensity of the exercise (Keytel *et al.*, 2005).

In terms of studies which have investigated energy intake responses to exercise-induced energy expenditure the use of direct calorimetry is not always feasible. The most commonly used techniques in studies of this nature are doubly labelled water (Whybrow *et al.*, 2008) which is costly and not without limitations, whole body indirect calorimetry (Horton *et al.*, 1994) and with regards to paediatric populations the FLEX (actual name) heart rate method (Moore *et al.*, 2004; Dodd, Welsman & Armstrong, 2008).

2.4.1.1 Doubly Labelled Water Technique for Assessing Energy Expenditure

Doubly labelled water measurement is typically described as being the gold standard technique for accurately assessing free-living energy expenditure (Eston, Rowlands & Ingledew, 1998). Murgatroyd, Shetty and Prentice (1993) summarise the technique, which involves each participant providing a baseline urine sample followed by the consumption of an oral dose of water containing stable isotopes ($^{2}H_2{}^{18}O$). Deuterium (^{2}H) labels the body's water pool and its rate of disappearance from the body (K_2) provides a measure of water turnover. Oxygen (^{18}O) labels both the water and bicarbonate pools and thus it's disappearance from the body (K_{18}) provides a measure of water and bicarbonate turn-over. Carbon dioxide (CO_2) production rate is therefore calculated as the difference between the two rate constants (K_{18}-K_2), which is then converted to energy expenditure using indirect equations and calculations.

The doubly labelled water technique has successfully been used in infants (Davies *et al.*, 1991) and is also well tolerated by children and adolescents between the ages of 7-11 years (Emons *et al.*, 1992), 7-15 years (Livingstone *et al.*, 1992a) and 9.3±0.6 years (lean and obese) (Maffeis *et al.*, 1995). The doubly labelled water technique is a simple and non-invasive method for assessing free-living energy expenditure, requiring a minimum sampling period of 6-7 days where young people are concerned (Murgatroyd, Shetty & Prentice, 1993). However, this technique is also markedly more expensive compared to alternative means of quantifying energy expenditure, in terms of materials and continuous isotopic analysis (Spurr *et al.*, 1988), therefore limiting its use. It is also technically demanding and time-consuming during the analysis phase (Murgatroyd, Shetty & Prentice, 1993). Furthermore, the resultant data from the doubly labelled water technique is however simply an average energy expenditure value for each 24-hour period (Spurr *et al.*, 1988), providing no information regarding frequency, intensity and duration of any exercise performed during the sampling period (Murgatroyd, Shetty & Prentice, 1993).

2.4.1.2 Indirect Calorimetry

In contrast to direct calorimetry, indirect calorimetry relies on researchers assessing the amount of heat produced by the body indirectly, by measuring $\dot{V}O_2$ in relation to carbon dioxide $\dot{V}CO_2$, known as the respiratory exchange ratio or respiratory quotient (Murgatroyd, Shetty & Prentice, 1993). The respiratory exchange ratio differs depending on which mix of substrates is oxidised. Thus a respiratory exchange ratio of 1.00 implies that carbohydrate is the main energy source, whilst fat is the main energy source being oxidised when the respiratory exchange ratio is 0.70 (Elia & Livesey, 1992). Therefore, compared to direct calorimetry, indirect calorimetry provides a measure of carbohydrate and fat oxidation rates. With such information energy expenditure can then be estimated using the Weir equation (Weir, 1949), where RQ is the respiratory quotient:

$$\text{Energy Expenditure (Kcal X min}^{-1}) = \dot{V}O_2\ (3.9 + 1.1\ RQ)$$

Indirect calorimetry can be conducted using chambers, known as whole body indirect calorimeters, facemasks or mouthpieces and hoods or tents (Murgatroyd, Shetty & Prentice, 1993). Whole body indirect calorimeters have been well accepted by young people and have successfully been used in various studies to estimate energy expenditure. Bitar et al. (1996) and Treuth, Adolph and Butte (1998) used whole body indirect calorimetry successfully in children aged 10.5±0.5 and 8-12 years old, respectively, to estimate 24-hour energy expenditure. Similar to doubly labelled water, whole body indirect calorimeters are utilised to validate alternative means of quantifying energy expenditure in young people. The main justification for this is its accuracy and precision in quantifying young people's energy expenditure. Moon et al. (1995) evaluated the performance of four whole body indirect calorimeters in children (12.0±2.0 years old). Overall Moon et al. (1995) concluded that whole body indirect calorimeters accurately estimated individual estimates of $\dot{V}O_2$ and thus energy expenditure by ±3% with regards to variability. Emons et al. (1992) reported a similar discrepancy of 2% for estimating 24-hour energy expenditure using whole body indirect calorimetry, when validated against doubly labelled water in 7-11 year old children. Whole body indirect calorimetry therefore provides an accurate, precise and simple means of quantifying energy expenditure, in addition to information concerning substrate utilisation and activity patterns (Murgatroyd, Shetty & Prentice, 1993). However, since it requires young people to spend prolonged periods of time out of their natural environment, in artificial and restricted conditions, behavioural changes are likely to be induced (Emons *et al.*, 1992). This therefore restricts the use of whole body indirect calorimetry in free-living environments (Luke *et al.*, 1997).

Small, portable indirect calorimeters have been developed for use in the field, however their high cost, requiring careful design and expertise (Murgatroyd, Shetty & Prentice, 1993) and the need to wear cumbersome and obtrusive equipment over a 24-hour period or longer is likely to induce behaviour changes (Lamonte, Ainsworth & Tudor-Locke, 2003). Such equipment will also need to be validated in a field setting in order to be deemed as a valid technique for accurately assessing free living energy expenditure

(Lamonte, Ainsworth & Tudor-Locke, 2003) and so alternative techniques should be considered.

2.4.1.3 Heart Rate Monitoring and the FLEX Heart Rate Technique

Monitoring heart rate to assess free living energy expenditure in young people is a popular and effective method due to its practicality (Livingstone, Robson & Totton, 2000), compared to direct calorimetry, indirect calorimetry and doubly labelled water. This method utilises small, portable, watch type devices known as receivers and a strap worn around the chest, which transmits heart rate data to the receiver by telemetry. Heart rate can be sampled over 15, 30 or 60 second epochs and the data can be later downloaded onto a computer for analysis. When sampling at 60 second epochs, heart rate monitors can store substantial amounts of data (Spurr *et al.*, 1988; Bitar *et al.*, 1996). They are simple to use, relatively inexpensive (Maffeis *et al.*, 1995), robust, non-invasive (Treuth, Adolph & Butte, 1998), small, convenient and not cumbersome (Bitar *et al.*, 1996). Such qualities mean that the technique is popular and well tolerated by young people being the most socially accepted method for assessing free-living energy expenditure (Livingstone *et al.*, 1992a; Livingstone, Robson & Totton, 2000).

Subsequently, heart rate monitors have been successfully used with children as young as 4 years old, in both laboratory and field settings whilst the children completed various types of exercise (Treiber *et al.*, 1989). Bar-Or et al. (1996) reiterates this after simultaneously measuring heart rate using a heart rate monitor and ECG recordings in children aged 3-5 years. Treiber et al. (1989) and Bar-Or et al. (1996) concluded that polar heart rate monitors provide similar values to those of ECG readings and in children as young as 3 years are a valid method for assessing heart rate in resting, exercise and recovery situations. In terms of assessing heart rate during field based sport-specific exercise, heart rate monitors have successfully been used in habitually active adolescent girls aged 12-14 years old (Rumbold & Dodd, 2007). Bar-Or et al. (1996) also assessed social acceptability on two separate days, concluding that 90% of the time the children were ‘enthusiastic and positive’ or ‘agreed’ in their responses, with regards to wearing the heart rate monitors.

Assessing energy expenditure from heart rate is grounded by the work of Berggren and Christensen (1950) and is based on the premise that $\dot{V}O_2$ is acutely related to an increase in heart rate due to exercise (Murgatroyd, Shetty & Prentice, 1993). It has long been assumed therefore that during exercise there is a linear relationship between heart rate and $\dot{V}O_2$ (Christensen *et al.*, 1983) allowing energy expenditure to be quantified, using the predictive calculations of Weir (1949) for instance.

There lies two inherent limitations with using heart rate to estimate energy expenditure, which must be acknowledged and taken into consideration when conducting research and interpreting results. Firstly, at sedentary energy expenditure the linear relationship between heart rate and $\dot{V}O_2$ weakens (Livingstone, Robson & Totton, 2000). It is well documented that an individual's heart rate is affected and partially dissociated from energy expenditure by posture, physical and emotional state, the environment (ambient temperature and humidity), food intake, hydration status, fatigue and previous activity (Montoye & Taylor, 1984; Murgatroyd, Shetty & Prentice, 1993; Keytel *et al.*, 2005). This is not the case during exercise, where heart rate provides an indication of the relative stress imposed on the cardiopulmonary system (Murgatroyd, Shetty & Prentice, 1993). At very high levels of exercise near maximal capacity (Leonard, 2003), the relationship between heart rate and $\dot{V}O_2$ also becomes tenuous. However, during moderate to vigorous intensities of exercise the relationship between heart rate and $\dot{V}O_2$ is more secure (Livingstone, 1994). The relationship between heart rate and $\dot{V}O_2$ has high inter-individual differences, due to variations in long term variables such as fitness levels, cardiac stroke volume, haemoglobin content, local blood flow regulation, oxygen extraction (Li, Deurenberg & Hautvast, 1993), genetics (Leonard, 2003), body composition, illness and aging (Li, Deurenberg & Hautvast, 1993). To account for this, it has been established that participants should be individually calibrated for heart rate-$\dot{V}O_2$ relationship, after findings of inter-individual variability in the slopes of the regression line (Christensen *et al.*, 1983; Li, Deurenberg & Hautvast, 1993). Li, Deurenberg and Hautvast (1993) also suggest that individually calibrated heart rate-$\dot{V}O_2$ curves should be generated for specific exercise situations.

Spurr et al. (1988) developed the notion of the FLEX heart rate technique for assessing energy expenditure. This enables a predetermined heart rate threshold to be established for each participant, which differentiates between sedentary and activity heart rate (Livingstone, Robson & Totton, 2000). To identify this threshold, heart rate and $\dot{V}O_2$ are measured simultaneously during resting conditions, more specifically lying supine, sitting and standing and during standardised exercise protocols (Leonard, 2003). The FLEX heart rate threshold was defined by Ceesay et al. (1989) as the mean of the highest heart rate during rest and the lowest heart rate during the lightest imposed exercise. Thus, when heart rate falls below the FLEX heart rate threshold the mean of the $\dot{V}O_2$ values ascertained during the resting conditions are used to quantify sedentary energy expenditure, whilst for heart rate values above the FLEX heart rate, energy expenditure is determined based on the linear regression between heart rate and $\dot{V}O_2$ during the exercise (Livingstone *et al.*, 1992a).

With regards to young people, several validation studies have been conducted to determine the agreement between energy expenditure quantified using heart rate and energy expenditure quantified using a standard or reference technique such as DLW or indirect calorimetry. One of the first of these studies validated the FLEX heart rate technique against doubly labelled water in 36 free-living children, 19 boys and 17 girls, aged 7, 9, 12 and 15 years (Livingstone *et al.*, 1992a). Individual heart rate and $\dot{V}O_2$ regression lines were established after each child had rested for 20 minutes and were >2 hours post-prandial. The children were required to remain in each of the calibration activities for 6 minutes in total, consisting of a 3 minute equilibration period followed by a 3 minute sampling period. The resting calibration points included lying supine, sitting and standing. The two calibration points which fell above the FLEX heart rate threshold involved exercising on a treadmill at 2.7 $km \cdot h^{-1}$ at a gradient of 10% and then at 4.0 $km \cdot h^{-1}$ at a gradient of 12%. Within 7 days of the heart rate and $\dot{V}O_2$ calibrations, doubly labelled water was administered and urine samples collected for 10 or 14 days depending on the age of the child. Heart rate data was also recorded for 2-3 days. Overall, for all participants (n=36), when the heart rate method was compared to doubly labelled water, energy expenditure was underestimated by 3.4%, 8.86±2.02 and 9.15±1.77 $MJ \cdot d^{-1}$ respectively, with regards to the energy expenditure method employed. These results indicated that the methods showed good agreement when estimating group energy expenditure, reiterated by 95% confidence intervals of bias of -0.56 to +0.01 $MJ \cdot d^{-1}$. Limits of agreement, however were -1.99 to +1.44 $MJ \cdot d^{-1}$, with individual energy expenditure values ranging from -16.7% to +18.8% in 23 participants. However despite this heart rate energy expenditure estimates lay within ±10% of those estimated by doubly labelled water. On an individual basis there were more discrepancies in energy expenditure identified in the 7 and 9 year old age bracket compared to the 12 and 15 year old individuals, with values ranging from -6.1±10.5 and +0.4±7.2%, respectively. Only for the 15 year old age group did heart rate overestimate energy expenditure in comparison to the doubly labelled water estimates. Livingstone et al. (1992a) suggested that the return of heart rate to baseline levels may lag behind that of $\dot{V}O_2$ thus overestimating energy expenditure in this age group. Livingstone et al. (1992a) concluded that the FLEX heart rate technique is suitable for predicting group estimations of habitual energy expenditure in free-living children, however lacks precision for estimating the energy expenditure of individuals.

Interestingly, Livingstone et al. (1992a) reported that the heart rate method significantly underestimated energy expenditure when compared to doubly labelled water ($p<0.001$), in the 9 year old children, with percentage difference values ranging from -14% to -1.1%. In contrast, Maffeis et al. (1995) conducted a similar study but did not identify a significant underestimation of energy expenditure by the heart rate method in a group of 7 lean pre-pubertal children (3 boys, 4 girls) of a similar age (9.3±0.6 years old). Individual FLEX heart rate thresholds were established, using walking and running exercise on a treadmill to determine six calibration points above the FLEX heart rate thresholds. Heart rate data was collected over 2-3 school days, alongside 7 days of doubly labelled water. The results were favourable as energy expenditure assessed by the FLEX heart rate technique only overestimated energy expenditure values quantified by doubly labelled water by 0.2±7.8%, 8.43±2.02 and 8.42±2.30 $MJ \cdot d^{-1}$ respectively, with regards to the enrgy expenditure measurement technique employed. On a group level 95% confidence intervals for bias indicated good agreement between the two techniques of -0.59 to 0.63 $MJ \cdot d^{-1}$. At an individual level agreement was low, -1.30 to 1.34 $MJ \cdot d^{-1}$, with percentage difference values ranging from -4.1% to 12.4%. The sampling duration for heart rate of 2-3 days in the Livingstone et al. (1992a) study was consistent with that of Maffeis et al. (1995), thus inconsistent findings may have been due to inappropriate FLEX heart rate thresholds being established alongside unrepresentative calibration data (Livingstone *et al*., 1992a; Maffeis *et al*., 1995). Maffeis et al. (1995) suggested that the inclusion of only two calibration points above the FLEX heart rate threshold may have been responsible for the inconsistent findings. These results reiterate those of Livingstone et al. (1992a) that when predicting energy expenditure it is beneficial to collate group estimates as opposed to relying on individual sets of energy expenditure data. In addition, to establish accurate regression equations between heart rate and $\dot{V}O_2$ ample calibration points above the FLEX heart rate threshold are required for predictions of energy expenditure to be precise (Maffeis *et al*., 1995).

Indeed, it also seems that sampling heart rate over several days is required to further improve precision and accuracy when estimating energy expenditure in young people. Unlike Livingstone et al. (1992a) and Maffeis et al. (1995) who sampled heart rate over 2-3 days, a study by Emons et al. (1992) only monitored heart rate over 1 day. Emons et al. (1992) quantified energy expenditure using three methods simultaneously in 19 children (9 boys, mean age 9.3 years and 10 girls, mean age 8.1 years), namely heart rate, whole body indirect calorimetry and doubly labelled water. Results showed that the FLEX heart rate technique overestimated energy expenditure by 10.4% compared to whole body indirect calorimetry and by 12.3% compared to doubly labelled water. Increasing the number of days over which heart rate is sampled may improve the precision and accuracy of the assessment (Maffeis *et al.,* 1995). Livingstone et al. (1992a) and Maffeis et al. (1995) collected heart rate data over 2-3 days alongside doubly labelled water and obtained percentage differences of -3.4% and +0.2±7.8% respectively. Such results are more favourable compared to that of Emons et al. (1992) who obtained discrepancies of 12.3% over 1 day when energy expenditure assessed using the FLEX heart rate technique and the doubly labelled water technique were compared. It seems increasing the number of days heart rate data is collected over may be a contributory factor in improving group estimates of energy expenditure in free-living young people (Livingstone *et al.*, 1992a; Maffeis *et al.*, 1995). Emons et al. (1992) also concluded that the activities involved in the calibration of the individual heart rate and $\dot{V}O_2$ equations in young people should replicate as closely as possible those activities which are intended to be measured, in order to better assess energy expenditure. This principal has been demonstrated in adult populations (Ceesay *et al*., 1989).

Previous studies (Emons *et al*., 1992; Livingstone *et al*., 1992a; Maffeis *et al*., 1995) have not employed specific calibration activities and procedures when assessing energy expenditure in young people. In addition, heart rate-$\dot{V}O_2$ regression equations derived from laboratory calibration activities do not represent activities associated with free-living situations, thus Christensen et al. (1983) and Li, Deurenberg and Hautvast (1993) suggest that the number and type of calibration activities may affect the accuracy of estimating energy expenditure. Subsequently, it was suggested that in young people, representing usual spontaneous free-living activity patterns in the calibration activities may increase the precision and accuracy of energy expenditure estimates (Livingstone, Robson & Totton, 2000). Livingstone, Robson and Totton (2000) therefore investigated the affect of body postures and movements specific to that of young people to see how this influenced energy expenditure estimations. Individual heart rate-$\dot{V}O_2$ calibrations were derived from 7 boys aged 8-10 years old, whilst completing eight activities: lying supine, sitting, standing, arm and upper body exercises, stooping and twisting exercises, and continuous graded treadmill and cycling exercises. A 3 minute equilibration period was induced followed by a 3 minute sampling period. Heart rate was then monitored for >2 days, for 12 hours a day for each child, at a capture rate of 1 minute, within 2 weeks of the calibration procedure. Livingstone, Robson and Totton (2000) emphasise that under free-living conditions this calibration procedure should take place within 2 weeks of the actual measurement period. Livingstone, Robson and Totton (2000) derived 7 calibration equations from the 8 activities used in the calibration procedure. The results indicated that there was no significant difference in estimated energy expenditure of habitual activity patterns, derived from any of the regression equations. However, where it has been necessary to estimate the energy expenditure of specific exercise bouts such as cycling, it has been shown to be beneficial to replicate as closely as possible those activities which are intended to be measured (Ceesay *et al*., 1989). Livingstone, Robson and Totton (2000) also concluded that increasing the number of calibration activities may not be beneficial, since the whole procedure lasted for 2-2.5 hours. Since low coefficient of variation (%) in within and between subjects estimates of energy expenditure were obtained, it is debatable whether long, physically and mentally demanding procedures are optimal. Indeed, especially where young people are concerned compliance and motivation issues may become problematic, and therefore studies may favour calibration procedures which include ample calibration points (Maffeis *et al*., 1995) but not an extensive quantity of calibration points (Livingstone, Totton & Robson, 2000).

The FLEX heart rate technique is the only method of energy expenditure quantification which provides visually observable temporal representations into habitual activity patterns (Spurr *et al.*, 1988), associated cardio-respiratory function (Livingstone *et al.*, 1990), and an indication of the type, frequency and intensity of exercise performed and nature of day-to-day variability (Luke *et al.*, 1997; Hebestreit & Bar-Or, 1998). Such information is beneficial for intervention studies looking at the effects of exercise on acute energy intake. The first paediatric study of this nature was conducted by Moore et al. (2004) using 9-10 year old girls. The FLEX heart rate technique was used to establish the energy expenditure of several bouts of cycling exercise and overall energy expenditure for the waking hours of 1 day. This technique also enabled researchers to ensure the girls remained sedentary following the exercise bouts and also were inactive during a rest condition. Using a similar protocol, the FLEX heart rate technique has been used to monitor the energy expenditure of young girls (10-11 years) during the waking hours of 5 consecutive days on two separate occasions (Dodd, Welsman & Armstrong, 2008). The FLEX heart rate technique lends itself well to this type of research as it does not induce behavioural changes (Livingstone, Robson & Totton, 2000) or put participants in awkward situations or environments (Luke *et al.*, 1997) and thus affect on the findings. In this respect it is a versatile and feasible technique to use in sometimes difficult and remote field and free-living environments (Treuth, Adolph & Butte, 1998). Practical problems with heart rate monitors are inevitable when working with young people (Armstrong, 1998), however the plethora of benefits enable researchers to conclude that when assessing energy expenditure and physical activity in children heart rate monitoring is still the most popular field technique for this purpose (Livingstone *et al*., 1992a).

In summary, when energy expenditure estimated using heart rate was compared to data derived from doubly labelled water, values lay within ±10% of each other (Livingstone *et al.*, 1992a). Heart rate monitoring provides acceptable estimates of energy expenditure at a group level, however the efficiency of measurement of energy expenditure on an individual basis is limited (Livingstone *et al*., 1992a; Maffeis *et al*., 1995). Indeed, precision and accuracy of the energy expenditure measurement is enhanced by the establishment of an appropriate definition of the FLEX heart rate threshold for each participant, using ample calibration points in the active part of the calibration curve, to gain a representative relationship (Maffeis *et al.*, 1995). Livingstone, Robson and Totton (2000) do emphasise that excessive calibration points lead to long and time consuming protocols which may be plagued with compliance issues in young people. It seems that a minimum of three sampling days are required to obtain accurate and precise estimates of energy expenditure when using heart rate monitoring in young people (Livingstone *et al.*, 1992a; Maffeis *et al.*, 1995). Finally, the activities used in the individual calibrations must be representative of the activities which are likely to be monitored in the research (Ceesay *et al*., 1989; Emons *et al*., 1992). Therefore, if sport-specific exercise protocols are used, then the same sport-specific activities must be used during the FLEX heart rate calibration.

2.4.1.4 Accelerometry

Motion sensors work on the assumption that energy expenditure is closely related to the acceleration of the limbs and torso (Haskell *et al.*, 1993). Recently accelerometers have been used to assess acceleration, which is the change in velocity over time and as such, it quantifies the volume and intensity of movement (Freedson, Pober & Janz, 2005), thus providing information regarding intensity, frequency and duration of physical activity and exercise (Eston, Rowlands & Ingledew, 1998). These battery operated electronic devices provide activity counts based on the rate and displacement of the body’s centre of mass during movement (Lamonte, Ainsworth & Tudor-Locke, 2003), which are then calibrated with energy expenditure to give them biological meaning (Freedson, Pober & Janz, 2005). Accelerometers can be categorised depending on which plane of movement they measure acceleration (Harro & Riddoch, 2000). Specifically, uniaxial accelerometers are most sensitive to acceleration in the vertical plane, whilst omni-directional accelerometers measure acceleration in the vertical and medio-lateral plane, and triaxial accelerometers are sensitive to and measure acceleration in the vertical, medio-lateral and anterior-posterior directions (Rowlands & Eston, 2007). In terms of frequently used omni-directional, these include the Actical and with regards to triaxial accelerometers, the Tritrac or RT3 (Rowlands & Eston, 2007). The most frequently used uniaxial accelerometer in physical activity research is the Actigraph, of which there have been several different models used over the past decade (Corder *et al*., 2007; Rowlands & Eston, 2007).

One of the newest models at the time of writing, is known as the GT1M Actigraph (Actigraph™, LLC, Pensacola, Florida, USA), which is lightweight and compact, making this device ideal for use with young children and adolescents (Harro & Riddoch, 2000). Compared to previous generations and other omni-directional and triaxial accelerometers the GT1M has a battery life of over 14 days and the ability to store 1MB of activity data, which if sampling over 60 second epochs enables 1 year's worth of data to be collected. When sampling over 1 second epochs previous versions of the Actigraph and the available triaxial accelerometers could only collect activity data for 9 hours, whereas the GT1M 1MB memory enables data collection for 6 days in this mode (Rowlands & Eston, 2007). Such specifications means the GT1M Actigraph is ideal for use in field and free-living studies where the collection of energy expenditure over several consecutive days is necessary. In addition the ability of the GT1M to assess activity over 6 days, sampling over 1 second epochs is useful to track young people's spontaneous and sporadic habitual activity patterns (Fawkner & Armstrong, 2007; Rowlands & Eston, 2007).

The GT1M has been successfully used over seven consecutive days to assess free-living activity in 30 Indian adolescents (15.8±0.6 years old). Since there are several generations of the Actigraph being used to indirectly assess energy expenditure and physical activity in young people, Corder et al. (2007) investigated the comparability of activity data derived from the Actigraph Model 7164 and the newer GT1M model. Both accelerometers were worn simultaneously, with each placed centrally on either the right and left hip, during 7 days of free-living activity. Mean activity counts per minute were calculated for each monitor, allowing agreement between the data to be assessed using the Bland and Altman technique, Pearson correlation coefficients, paired t-test and regression analysis. Results indicated that there was a strong positive correlation between the accelerometers (r=0.95), which was significant (p=0.009), with Bland and Altman plots providing no evidence of heteroscedasticity. The GT1M version did however measure more counts per minute, by an average of 7% (372.9±19.5 and 347.9±19.0 $1 \cdot min^{-1}$, p=0.0121). These results suggest that the activity data from the two accelerometers is comparable, however if being directly compared the authors suggest that a correction factor of 0.928 is employed (Corder *et al*., 2007). With an increased memory capacity (Rowlands & Eston, 2007) and reduced inter-monitor variability (Rothney *et al*., 2008) the GT1M is an appropriate accelerometer to use for collecting activity data over several consecutive days. This device should be used solely and not in conjunction with any other accelerometer device, ideally.

Therefore, since the GT1M activity counts are highly correlated with those of previous models of accelerometers, validation studies involving these previous models can be used to provide an indication of the validity of the GT1M to assess energy expenditure in children and adolescents. However, Rowlands and Eston (2007) state that these activity counts cannot be directly compared between accelerometers of different types. Consequently, activity counts are typically calibrated with energy expenditure in order for activity information from different accelerometers to be compared and also to give the counts biological significance (Freedson, Pober & Janz, 2005). Specifically for the Actigraph, in children (Puyau *et al*., 2002) and adolescent girls (Treuth *et al*., 2004), count thresholds have been developed which enable the amount of time spent at different energy expenditure intensities (including sedentary) to be determined (sedentary, light, moderate and vigorous). Despite the ease of use of such count thresholds, Rowlands and Eston (2007) emphasise that when utilising count thresholds researchers must have some insight into the activities used to develop them, in order to avoid inaccuracies in energy expenditure estimation. In one instance, Eisenmann et al. (2004) used the Actigraph to estimate the energy expenditure of 11.4±0.4 year old children completing self-paced sweeping, bowling and basketball activities. Energy expenditure however was underestimated since Eisenmann et al. (2004) used treadmill-based prediction equation to calculate the energy expenditure imposed by the sweeping, bowling and basketball activities (Trost *et al*., 1998).

Typically therefore, accelerometer counts have been deemed to provide valid estimates of energy expenditure in young people when the calibration activities used are representative of the subsequent activities, which are to be employed in the research. In a recent review article, Plasqui and Westerterp (2007) concluded that out of those accelerometers which are commercially available, the CSA and MTI Actigraphs correlated well with doubly labelled water assessed energy expenditure in children and adolescents. In young people, the validity of Actigraph accelerometers has been scrutinised under laboratory conditions (Trost *et al*., 1998), during representative physical activities (Puyau *et al*., 2002; Schmitz *et al*., 2005) and in free-living environments (Ekelund *et al*., 2000).

During laboratory based treadmill walking and running Trost et al. (1998) assessed the energy expenditure of 30 children and adolescents (10-14 years old) using two CSA Actigraph accelerometers one positioned over the right hip and the other over the left hip, whilst also assessing $\dot{V}O_2$ via indirect calorimetry as a criterion measurement. There was a strong significant correlation between the activity counts from both monitors and energy expenditure assessed using indirect calorimetry (r=0.86 and 0.87, $p<0.001$). The authors concluded that in children and adolescents aged 10-14 years old it is sufficient for them to wear only one accelerometer over the hip, in order to obtain a valid measure of energy expenditure during treadmill walking and running. Such hip placement of the accelerometer is expected to be advantageous in field or free-living environments, since if worn on the wrist, Trost et al. (1998) suggest that the children and adolescents may also be inclined to tamper with the monitor and there may be an increased likelihood of extraneous arm movements being captured.

Puyau et al. (2002) support Trost et al. (1998) in terms of the hip placement of the CSA Actigraph accelerometer being optimal for estimating energy expenditure. Accelerometer derived energy expenditure, with one monitor worn on the right hip, was validated against indirect calorimetry over 6-hours whilst 26 children and adolescents (6-16 years old) completed a resting metabolic rate measurement and appropriate physical activities which were habitually representative, such as playing on the Nintendo, participating in arts and crafts, performing an aerobic warm-up, Tae Bo, treadmill walking, treadmill running and various other games. The hip mounted CSA Actigraph mean correlation between activity counts and energy expenditure derived from indirect calorimetry, for all participants was r=0.66±0.08. Puyau et al. (2002) concluded that the CSA Actigraph reflects energy expenditure during activity and although activity counts can be used to derive sedentary, light, moderate and vigorous thresholds, regression equations do not necessarily predict energy expenditure accurately or as well as individual calibration, since the accuracy is mostly dependent on the similarity of the type of activity and whether the activities are representative of the activities being imposed in exercise and appetite studies (Eisenmann *et al*., 2004). In terms of accelerometer placement, although the leg-mounted accelerometer provided more accurate estimates of energy expenditure, the children and adolescents experienced discomfort more so than with the hip placement, leading the authors to conclude that since accelerometers work on the premises of body acceleration and the mass of the body displaced, placement as near to the centre of mass is recommended (Puyau *et al*., 2002). Trost, McIver and Pate (2005) reiterate that accelerometers should be placed on the hip or lower back.

Several studies have successfully used, and others validated, the use of uniaxial accelerometers in free-living environments to assess the energy expenditure of children and adolescents. The CSA Actigraph accelerometer has been used in adolescent girls aged 11-12 years old to assess free-living energy expenditure over 6 days (weekend and weekdays) (Treuth *et al*., 2007). Similarly the GT1M version has also been used to assess free-living energy expenditure over 7 days in adolescent boys and girls (15.8±0.6 years old) (Corder *et al*., 2007). In terms of reliability, it has been suggested that a minimum of ≥4 days of activity monitoring is required to provide a reliable estimate of energy expenditure in children and adolescents (7–15 years old) (Janz, Witt & Mahoney, 1995).

The validity of the CSA Actigraph accelerometer for assessing energy expenditure under free-living conditions in adolescent athletes (speed skaters) has also been explored (Ekelund *et al*., 2000). Seven male athletes took part in the study (18.2±1.1 years old) and were monitored twice over two 10 day periods, 5 months apart, once during off season and once during pre-season. The off season training involved mainly running, whilst the pre-season encompassed mainly inline skating, in addition to circuit training, slideboarding, weight training, skating imitations and on a few occasions running and cycling. For the first 8 consecutive days during each 10 day period, physical activity was monitored using the CSA Actigraph accelerometer positioned at the lower back, as close to the centre of gravity as possible since activity counts are based on the rate and displacement of the body's centre of mass during movement (Lamonte, Ainsworth & Tudor-Locke, 2003). Minute-by-minute heart rate was also sampled for the first 8 days, whilst doubly labelled water was employed to determine total daily energy expenditure for the full 10 days during each period. Activity counts, heart rate and $\dot{V}O_2$ were simultaneously measured during five calibration activities which included 6 minutes walking at 4.5 $km \cdot h^{-1}$, 6 minutes walking at 6.5 $km \cdot h^{-1}$, 6 minutes running at 10 $km \cdot h^{-1}$, 6 minutes cycling at 120 W and 6 minutes cycling at 180 W. Results demonstrated that activity counts were significantly correlated to energy expenditure derived from doubly labelled water during the off season ($r=0.90$-0.96, $p<0.01$) however this was not the case during the pre-season ($r=0.32$-0.57). Ekelund et al. (2000) suggested that such discrepancies in energy expenditure estimates between seasons may be due to the CSA Actigraph accelerometer being most sensitive to vertical accelerations, inherent in running but not in skating activities. Since 72% of time was spent running during the off season compared to only 6% during the pre-season, the authors concluded that energy expenditure was better reflect during the off season due to this. Thus, decreased vertical movement of the body during activities such as cycling, weight training, inline skating and slideboarding inherent in the pre-season training resulted in energy expenditure being underestimated (Ekelund *et al*., 2000; Ekelund *et al*., 2004; Corder *et al*., 2007). With the physical activity or exercise that is likely to be assessed in mind, these results indicate that the correct accelerometer in terms of the sensitivity to the appropriate movement planes must be selected. Certainly this study demonstrates that the type of physical activity or exercise has an influence when estimating energy expenditure, and consequently is beneficial for the activities used in the calibration process to represent those activities likely to be used in the subsequent research, as previously highlighted (Eisenmann *et al*., 2004). With regards to sedentary periods and walking activities Ekelund et al. (2000) concluded that the activity counts explained a considerable amount of the variation in daily energy expenditure and thus are appropriate for assessing free-living energy expenditure.

2.4.1.5 Simultaneous Measurement of Heart Rate and Physical Activity

It is well documented that heart rate is affected by a number of external variables such as posture, physical and emotional state, the environment (ambient temperature and humidity), energy intake, hydration status, fatigue and previous physical activity (Montoye & Taylor, 1984; Murgatroyd, Shetty & Prentice, 1993; Keytel *et al.*, 2005). Therefore heart rate monitors are unable to differentiate between increases in heart rate as a result of exercise or the parameters identified above (Rennie *et al.*, 2000). It is acknowledged that the return of heart rate to baseline levels may lag behind that of $\dot{V}O_2$, thus overestimating energy expenditure, which has been demonstrated to be the case in young people (Emons *et al*., 1992; Livingstone *et al.*, 1992a; Maffeis *et al.*, 1995; Livingstone, Robson & Totton, 2000). The tenuous relationship between heart rate and $\dot{V}O_2$ at low intensities of energy expenditure, enables factors such as anxiety and increases in body temperature to increase heart rate without an associated increase in $\dot{V}O_2$ and therefore energy expenditure (Livingstone, Robson & Totton, 2000). It is also acknowledged that uniaxial accelerometers underestimate energy expenditure during cycling and upper-body movement (Ekelund *et al*., 2000; Ekelund *et al*., 2004; Corder *et al*., 2007). A solution to such inaccuracies in energy expenditure estimation has shown potential in the form of simultaneous heart rate and motion monitoring (Brage *et al*., 2004). In turn this may improve the estimation of energy expenditure compared to using heart rate data or motion data alone (Luke *et al.*, 1997; Brage *et al*., 2004).

Eston, Rowlands and Ingledew (1998) investigated this hypothesis in 30 children (15 boys and 15 girls), aged 8-11 years old. Activities included in the calibration process were representative of young people's habitual activities, such as walking at 4 and 6 km·h^{-}

^{1}, running at 8 and 10 km·h^{-1}, hopping, catching and sitting crayoning. The children spent 4 minutes performing each activity, with the exception of sitting crayoning which they spent 10 minutes performing. During these periods heart rate was monitored and a triaxial accelerometer (Tritrac-R3D) sampling over 60 second epochs assessed activity, a uniaxial accelerometer (WAM accelerometer) sampled activity over 60 second epochs and a pedometer provided a count of total movements. Oxygen uptake was assessed via indirect calorimetry, averaged over 30 seconds, which was used as a criterion measure. The results indicated that the best single predictor of energy expenditure was the triaxial accelerometer, accounting for 82.5% of the variance, whilst heart rate and the uniaxial accelerometer contributed to 63.8% and 60.9% respectively. When two measures were combined, the best two predictors were the triaxial accelerometer (contributing 82.9% to the energy expenditure estimate) and heart rate (contributing 2% to the energy expenditure estimate) equating to 85% of the variance in total. Although heart rate only made a small, but significant (p<0.01) contribution to the variance. In terms of the combination of heart rate (65.5%) in conjunction with the uniaxial accelerometer (7.7%), 73.2% of the variance was accounted for. Eston, Rowlands and Ingledew (1998) concluded that since heart rate only contributed slightly to the variance when combined with the triaxial accelerometer the additional cost and labour is not warranted. Although not identified by the authors, it seems however from these findings that when heart rate is the main contributor to energy expenditure quantification in young people, the accompaniment of this with a less expensive uniaxial accelerometer is warranted to better estimate energy expenditure. This is especially the case considering the improvements in the specification of the recently released uniaxial GT1M Actigraph. It is the only commercially available accelerometer which has the ability to sample at 60 second epochs for 1 year and at 1 second epochs for 6 days (Rowlands & Eston, 2007) as well as being small and compact. Such qualities make the GT1M very appealing for use with young people and certainly ideal for assessing free-living energy expenditure over several consecutive days. Overall, Eston, Rowlands and Ingledew (1998) concluded that the combined use of heart rate and activity was an acceptable method for estimating energy expenditure in young people whilst they complete appropriate physical activities, not only for groups of children but for individual children as well.

2.4.2 Resting Metabolic Rate versus Basal Metabolic Rate

Exercise-induced energy expenditure data, assessed using the techniques previously discussed, can be combined with a measure of basal or resting metabolic rate in order to provide an estimation of 24-hour energy expenditure (Livingstone, Robson & Totton, 2000). The terms basal metabolic rate and resting metabolic rate are frequently, but incorrectly used interchangeably. Due to the energy cost of arousal levels, basal metabolic rate is slightly elevated above the metabolic rate during sleep (Goran & Astrup, 2002). Basal metabolic rate measurements require participants to have fasted for 10-12 hours and thus are said to be in a post-absorptive state. The participant must stay overnight in a research laboratory, where they are woken in the morning in the same room ready for the basal metabolic rate measurement to be conducted whilst they are in a rested state having not experienced any physical or psychological stress (Figueroa-Colon *et al.*, 1996). Such conditions are not readily available in research laboratories and have proved to be expensive, time consuming and inconvenient for participants and researchers (Figueroa-Colon *et al.*, 1996). Figueroa-Colon et al. (1996) also state that unfamiliar surroundings could induce sleep problems for young people, thus elevating basal metabolic rate. Consequently, these basal metabolic rate measurements tend to be performed in clinical environments and are thus inappropriate for use with young people in a field setting. A more appropriate method for young people would be resting metabolic rate measurements to take place whereby participants report to the laboratory in the morning, after a period of fasting (Figueroa-Colon *et al.*, 1996). Resting metabolic rate is ~3% higher than basal metabolic rate as it is assessed in a waking state (Goran & Astrup, 2002), where some physical activity has been performed prior to the measurement, mainly in the form of walking to reach the testing location.

Figueroa-Colon et al. (1996) compared whether staying overnight versus attending laboratories in the morning had an influence on resting metabolic rate measurements. The resting metabolic rate of 19 healthy, pre-pubertal girls (7.8±1.2 years old) was established on two different visits separated by 6 weeks, on three consecutive mornings each time. The procedure involved a 12-hour fast, followed by a 15 minute rest and then finally a 30 minute resting metabolic rate measurement period. The first measurement was where the children visited the laboratory in the morning followed by a resting metabolic rate measurement. The second and third measurements were where the children stayed overnight in the laboratory and the basal metabolic rate measurement was conducted in the morning. This procedure was repeated for visit 2. Analysis of variance demonstrated that there was no significant difference between the conditions for resting metabolic rate measurements, illustrated by the mean (±SD) during visit one and visit two, 1.06±0.13 and 1.09±0.10, 1.10±0.09 and 1.10±0.10 $MJ \cdot d^{-1}$, respectively. The mean coefficient of variation in intra-individual resting metabolic rate was calculated as 5.8% within children (range:

1.9-9.9%). These results suggest that in young girls a single resting metabolic rate measurement where they visit the laboratory in the morning is satisfactory, leading Figueroa-Colon et al. (1996) to conclude that to obtain a reliable measurement of resting metabolic rate participants do not need to stay over night in a laboratory setting. Bandini et al. (1995) reiterate that it is not essential or even necessary for participants to stay overnight in order to obtain an accurate and reliable metabolic measurement.

Typically, resting metabolic rate is measured under post-absorptive (fasted) conditions, although several studies in young people have assessed post-prandial resting metabolic rate (Firouzbakhsh *et al*., 1993; Goran, Kaskoun & Jackson, 1994; Morrison *et al*., 1996). It is important to know how such discrepancies in the pre-testing environment affect subsequent resting metabolic rate measurements in young people, especially when interpreting results of research studies. Goran and Nagy (1996) investigated the difference between post-absorptive resting metabolic rate and post-prandial resting metabolic rate in 19 children aged 4-9 years. The first visit involved an overnight stay in the research laboratory, and subsequently basal metabolic rate was measured in the morning after a 12-hour fast, and was identified as the post-absorptive trial. Two weeks later a resting metabolic rate measurement was conducted where the children consumed their normal breakfast at home prior to the assessment, thus this was identified as the post-prandial trial. Paired t-tests demonstrated that the metabolic rate values were significantly higher ($p<0.001$) by 11% under the post-prandial condition when compared to the post-absorped condition, 4.89 ± 0.63 versus 4.41 ± 0.63 $MJ\cdot d^{-1}$ respectively. Goran and Nagy (1996) suggested that the elevated resting metabolic rate following the post-prandial conditions was a consequence of the thermic effect of the food, known as diet-induced thermogenesis, which has been shown to elevate resting metabolic rate by 10-12%, 60-120 minutes following food ingestion (Maffeis *et al*., 1993b). Therefore, this procedure does not provide an accurate measure of resting metabolic rate but instead a measure of resting metabolic rate in conjunction with diet-induced thermogenesis. Such an elevation in resting metabolic rate due to diet-induced thermogenesis is however influenced by the calorific content of the meal consumed, and the period of time the resting metabolic rate measurement is taken following food ingestion (Maffeis *et al*., 1993b). Consequently, measuring resting metabolic rate post-prandially in young people introduces an array of additional extraneous variables, which are difficult to standardise between participants, and most importantly between research studies, in order for them to be comparable.

Taken together these findings suggest that post-absorped, resting metabolic rate measurements where participants visit the laboratory in the morning are most appropriate when working with young people. Thus the following section will outline studies and information pertaining to post-absorped, resting metabolic rate measurements where young people visit the laboratory in the morning as opposed to staying overnight.

2.4.2.1 Measurement of Resting Metabolic Rate

Due to the logistical constraints associated with assessing free-living energy expenditure via direct calorimetry and indeed its high expense (Murgatroyd, Shetty & Prentice,1993), resting metabolic rate is typically determined using pre-determined prediction equations (Goran & Astrup, 2002) or measured via indirect calorimetry (Rodriguez *et al*., 2000; McDuffie *et al*., 2004). There are an array of prediction equations that have been developed, which use variables such as weight, height, gender and age to provide an estimation of resting metabolic rate, thus avoiding the need to conduct a direct measurement (Molnàr *et al*., 1995). An accurate measure of resting metabolic rate is important when assessing 24-hour energy expenditure as it accounts for a large proportion of total daily energy expenditure in humans (Figueroa-Colon *et al*., 1996; Wong *et al*., 1996). Thus, in young people it is vital to know how resting metabolic rate values obtained via prediction equations compare to values derived from indirect calorimetry, which is often used as a criterion technique.

Validation studies in young people, comparing resting metabolic rate derived from prediction equations which indirectly measured resting metabolic rate, have found that prediction equations generally overestimate resting metabolic rate. Molnàr et al. (1995) assessed the resting metabolic rate of 371 10-16 year old pre-pubertal and post-pubertal Eastern European young people (boys and girls, lean and obese). Resting metabolic rate measured via indirect calorimetry was compared to resting metabolic rate predicted from five equations typically used in young people. The prediction equations include two by the World Health Organisation (1985), one by Robertson and Reid (1952), one by Fleisch (1951) and one by Boothby, Berkson and Dunn (1936). The resting metabolic rate of 119 lean girls was measured in a laboratory setting and was found to be 5.11±0.63 $MJ \cdot d^{-1}$. Analysis of variance demonstrated that the prediction equations produced by the World Health Organisation (1985) and Boothy, Berkson and Dunn (1936) all significantly ($p<0.001$) overestimated resting metabolic rate (8.0±9.9, 8.7±10.0 and 20.0±9.6%, respectively). The remaining two equations by Robertson and Reid (1952) and Fleisch (1951) also overestimated the resting metabolic rate in lean girls, although they did not reach statistical significance, the percentage differences were still high, 12.2±9.7 and 13.8±9.4%, respectively. Robertson and Reid (1952) and Fleisch (1951) suggested that the large standard deviation scores and range of differences between predicted and indirectly measured resting metabolic rate, demonstrates the high degree of individual error when attempting to predict resting metabolic rate. Therefore the measurement of resting metabolic rate via indirect calorimetry is preferable in comparison to predictive equations in young lean and obese boys and girls (10-16 years old). Rodriguez et al. (2000) reiterate the findings of Molnàr et al. (1995) that in individual children and adolescents (7.8–16.6 years old) the indirect measurement of resting metabolic rate is superior in comparison to prediction equations.

There have been several suggestions as to why discrepancies are apparent between predicted resting metabolic rates and indirectly assessed resting metabolic rate in young people. The equations used to predict resting metabolic rate were developed in the first half of the 20th century (Molnàr *et al*., 1995; Wong *et al*., 1996 ; McDuffie *et al*., 2004). Subsequently, the applicability of such equations to assess the resting metabolic rate of today's population (adults and young people) is questionable in terms of their generalizability and representativeness. A possible reason for this is differences in the types of calorimerty techniques used to develop such prediction equations in the early part of the century (Molnàr *et al*., 1995; Rodriguez *et al*., 2000). When using prediction equations to estimate resting metabolic rate knowledge about the demographics of the population that they were developed in must be considered and thus results interpreted appropriately. For instance, the prediction equations developed by Robertson and Reid (1952) and Boothby, Berkson and Dunn (1936) utilised the Du bois body surface area equation (Du Bois & Du Bois, 1916), which has only been validated in adults not young people, thus it's applicability to children and adolescents is questionable (Wong *et al*., 1996).

Body mass, height, age, body fat percentage and gender were the main variables used to develop the established prediction equations (Maffeis *et al*., 1993a; Molnàr *et al*., 1995; Wong *et al*., 1996; McDuffie et *al*., 2004) frequently used in young people to estimate resting metabolic rate. The onset of puberty occurs at a younger age than in previous decades, thus body mass is higher at an earlier age due to weight gain during puberty (Molnàr *et al*., 1995). This is further intensified by changes in nutrition, lifestyle and physical activity levels, which impact on body composition (Molnàr *et al*., 1995). Indeed, fat-free mass and fat mass change rapidly during puberty (Molnàr & Schutz, 1997), although such variables are not considered in the available prediction equations (Dietz, Bandini & Schoeller, 1991). Molnàr and Schutz (1997) state that the major determinant of resting metabolic rate in lean and obese children and adolescents is fat-free mass. Using indirect calorimetry Molnàr and Schutz (1997) measured the resting metabolic rate of pre and post-pubertal boys (116 lean, 77 obese) and girls (119 lean, 59 obese) aged 9-17 years old. Fat-free mass and fat mass were assessed using skinfold measures (triceps, biceps, supra-iliac, sub-scapular and calf). For the whole cohort of children and adolescents the main determinant of resting metabolic rate was found to be fat-free mass, which accounted for 79.8% of the variance in resting metabolic rate. Age, gender and fat mass added a further 3.8, 1.1 and 0.8% respectively, however pubertal development had no influence on resting metabolic rate. Therefore, when using prediction equations, the body composition of the population they were developed in is important. For example the prediction equations by the World Health Organisation (1985) were developed using 3-18 year old young people from underdeveloped and developed countries (Rodriguez *et al*., 2000). According to Wong et al. (1996) approximately 45% of the cohort were young and physically active. Thus it is reasonable to expect that when such equations are used in sedentary participants, they are likely to overestimate resting metabolic rate (Wong *et al*., 1996) mainly due to individual discrepancies in amounts of fat-free mass. On this basis Rodriguez et al. (2000) suggest that fat-free mass and age specific prediction equations are required.

In conclusion, although prediction equations have been cross-validated in several different paediatric populations (Rodriguez *et al*., 2000), this has not yet been the case for adolescent habitual exercisers. There are also large individual discrepancies between predicted and indirectly measured resting metabolic rate in young people, thus indirect assessment is recommended in order to obtain an accurate and representative measurement (Molnàr *et al*., 1995; Rodriguez *et al*., 2000).

2.4.2.2 Equipment and Protocol for the Measurement of Resting Metabolic Rate

Where young people are concerned, there appears to be a lack of consistency in terms of suitable equipment and an appropriate protocol to use for indirectly measuring resting metabolic rate. Equipment and protocols used to assess resting metabolic rate in young people have previously been based on those used with adult participants.

The equipment used to collect a gas sample during a resting metabolic rate measurement typically takes the form of a mouthpiece and nose clip, which the participants are required to wear for the duration of a 40 minute measurement period (Livingstone *et al.*, 1990). However, when choosing a method to assess resting metabolic rate in young people, the demands placed on the child or adolescent have to be considered, since it would not be appropriate if the chosen technique induced any distress (Goran & Astrup, 2002). Since it is imperative that young people remain still and relaxed for the duration of the measurement period, Livingstone et al. (1990) suggested that facemasks, mouthpieces and nose clips are unsuitable for use with young people. They are likely to induce un-physiological breathing resulting in a stress-induced tachycardia, which may in turn provide unrealistic and inaccurate values (Livingstone *et al*., 1990). A ventilated hood can be used to assess resting metabolic rate and has been deemed a more appropriate technique to be used with young people. Murgatroyd, Shetty and Prentice (1993) outline the basic technique, which involves the participant sitting or lying quietly with a transparent canopy or hood over their upper body. This ventilated hood technique is ideal for use with young people since it is not as cumbersome, inconvenient or uncomfortable as facemasks, mouthpieces and nose clips. It is also likely to provide a more accurate representation of resting metabolic rate in this special population.

In terms of an appropriate protocol to use, the main concern is the duration of the resting metabolic rate measurement period. In paediatric populations, measurement durations for resting metabolic rate protocols have varied between 5 minutes and 60 minutes (Ventham & Reilly, 1999). The use of long duration measurement periods is frequently evident in the literature when resting metabolic rate has been assessed in children and adolescents (Molnàr *et al.*, 1995; Molnàr & Schutz, 1997; Rodriguez *et al.*, 2000; McDuffie *et al*., 2004). Although young people have accepted long duration protocols, often procedures have had to be put into place to ensure that young people remain still and relaxed for the full duration of the measurement. Typically, this has involved young people watching cartoons on television or listening to music and storybooks in order to promote compliance (Molnàr *et al.*, 1995; Molnàr & Schutz, 1997; McDuffie *et al*., 2004). Amorim, Byrne and Hills (2007) demonstrated that in fourteen 8-12 year old young people, there was no significant difference in indirectly assessed resting metabolic rate when conducted whilst the children watched television compared to without television. Regardless of this, long duration protocols are notorious for increasing the likelihood of coughing, sneezing and fidgeting to occur, which has to be accounted for in subsequent data analysis and is likely to affect the accuracy of the resting metabolic rate measurement (Jackson, Pace & Speakman, 2007).

When assessing the resting metabolic rate of young people, Ventham and Reilly (1999) suggest that it may be justifiable to employ shorter and simpler protocols in terms of the measurement duration. This is further supported by the inherent disadvantages with using long duration protocols and no evidence to suggest that such lengthy protocols are more accurate that short duration protocols. Although short duration protocols have not been directly compared to long duration protocols in young people there is some evidence in young children that short duration protocols produce more repeatable results. Jackson, Pace and Speakman (2007) assessed the resting metabolic rate of 11 preschool children (4 girls, 7 boys) aged 4.2±1.0 years old, on three occasions over a 2 week period. The protocol involved an overnight fast followed by a 20-25 minute measurement period using the ventilate hood technique. The results indicated that there was no significant difference between resting metabolic rate measurements over the days of measurement, with a coefficient of variation of 6.8%. Jackson, Pace and Speakman (2007) demonstrated that even shorter measurement periods of 10-15 minutes produced more favourable coefficient of variation results. By excluding the first 10 minutes of the measurement period and periods of large activity (coughing, sneezing etc), which are inherent problems when using longer protocols with young people, the coefficient of variation was on average 4% lower, equating to 2.8%. Jackson, Pace and Speakman (2007) concluded that only a single, short measurement of resting metabolic rate is required to obtain a reliable estimate in young people. Such an advantage of excluding the first 10 minutes of the measurement period and any periods of large activity, suggests that there is no benefit in using long duration resting metabolic rate protocols, over short duration protocols, with young people.

The findings of Ventham and Reilly (1999) supported those of Jackson, Pace and Speakman (2007), however they proposed a short duration protocol which further improved the coefficient of variation. Ventham and Reilly (1999) aimed to determine the reproducibility of a short duration resting metabolic rate measurement using outpatient conditions in young people. Nine boys and 9 girls aged 6.4–11.6 years old, took part in the study. Following an overnight fast (10–12 hours) and a 5-10 minute lying supine period, resting metabolic rate was assessed using the ventilated hood technique. The protocol consisted of a 5-10 minute 'settling in' period and once a 'steady state' in $\dot{V}O_2$ was achieved, a 12-16 minute measurement was taken. To ensure the children lay quietly they were allowed to listen to music or story tapes. To assess the reproducibility of the resting metabolic rate measurement, three measurements were made on alternate days within the same week. The results showed there to be no significant differences in resting metabolic rate between days (p=0.26) and the mean coefficient of variation for intra-individual resting metabolic rate was 2.6±1.7% (range: 0.1-7.2%). The results of Ventham and Reilly (1999) support those of Jackson, Pace and Speakman (2007) in that a short duration resting metabolic rate measurement provides highly reproducible results. Taken together these results suggest that long duration resting metabolic rate protocols are unnecessary in young people, since reproducible results can be obtained using short and simple protocols, which are practical for the research team and attractive for the participants (Ventham & Reilly, 1999; Jackson, Pace & Speakman, 2007).

In summary, resting metabolic rate measurements assessed when the participant is in a post-absorped state, and visits the laboratory in the morning, is a more practical and thus more appropriate method for use with young people, and can also be used in a field setting. The need for participants to remain overnight in the research laboratory to obtain an accurate and reliable measurement of resting metabolic rate is unnecessary (Bandini *et al.*, 1995; Figueroa-Colon *et al.*, 1996). When assessing resting metabolic rate in individual young people, indirect measurement produces more accurate and representative results in comparison to prediction equations (Molnàr *et al.*, 1995; Rodriguez *et al*., 2000). The ventilated hood technique is the most appropriate method to use when assessing resting metabolic rate in young people, as nose clips and mouthpieces may cause distress thus affecting the result (Livingstone *et al*., 1990). Short duration protocols do not seem to be any less accurate than long duration protocols, providing a valid estimate of resting metabolic rate in young people (Ventham & Reilly, 1999; Jackson, Pace & Speakman, 2007). Short duration protocols promote compliance and are more practical for young people and researchers (Ventham & Reilly, 1999). It seems the protocol proposed by Ventham and Reilly (1999) is an appropriate procedure to follow when working with children and adolescents due to its high reproducibility.

2.4.3 Summary Energy Expenditure Assessment

The FLEX heart rate technique appears to be the most appropriate method to employ when an estimation of free-living energy expenditure is required in adolescent populations. Coupled with a simultaneous measurement of physical activity (accelerometry) where equipment is available, an even more sensitive method of energy expenditure estimation can be established. Short duration resting metabolic rate procedures are also beneficial when working with paediatric groups as opposed to the prediction of this parameter, which may become increasingly problematic when using trained adolescent participants.

Appropriate techniques need to be chosen carefully in order to assess energy expenditure as accurately as possible in young people involved in exercise and appetite studies. By addressing the logisitics of the research, such as the age of the participants along with the study time frame, appropriate energy expenditure assessment methods can be decided upon. The chosen, justifiable methods can then be utilised in exercise and appetite studies to explore energy intake compensation for exercise-induced energy expenditure.

2.5 Short-Term Energy Intake, Macronutrient Selection, Subjective Appetite and Ghrelin Concentrations following Exercise

The final section of this literature review will outline studies which have explored intake, subjective appetite and ghrelin concentrations following exercise, in paediatric groups. Where possible in the following outline only studies specific to paeditiric populations will be discussed. Where there is no paediatric data available, sport-specific information from adult studies will be reported and in particular findings from adult females. With regards to ghrelin data, information regarding sport-specific protocols will again be outlined and also information regarding adult females since at present there is no data available for children or adolescents. For acylated ghrelin concentrations following exercise, studies involving adult males will be discussed as there is no data for paediatric groups or adult females.

2.5.1 Energy Intake Compensation for Previous Energy Intake

Early work with regards to appetite and energy intake compensation in young people mainly concentrated on the impact of dietary manipulations on subsequent energy intake. There is a lack of information with regards to energy intake compensation for food preloads in adolescent populations and moreover in trained adolescent populations. Studies are limited to pre-school children (Birch & Deysher, 1986; Birch, McPhee & Sullivan, 1989; Wilson, 1991; Birch *et al*., 1993; Wilson, 1994; Hägg *et al*., 1998; Wilson, 1999; Hetherington, Wood & Lyburn, 2000), in which the findings are equivocal. Several of these studies have not identified either a compensation or down-regulation in energy intake at a test meal following a previous energy dense preload (Wilson, 1991; Wilson, 1994; Hägg *et al*., 1998; Wilson, 1999). In contrast, other studies have identified a down-regulation in energy intake which matched the energy content of the preload (Birch & Deysher, 1985; Birch & Deysher, 1986; Birch, McPhee & Sullivan, 1989; Hetherington, Wood & Lyburn, 2000), with two studies identifying a partial down-regulation (Birch *et al*., 1993; Hägg *et al*., 1998).

More recent studies in children aged 4-6 years old (Zandstra *et al*., 2000) 6-9 years old (Cecil *et al*., 2006), 9-12 years old (Warren, Henry & Simonite, 2003) and 5-12 years old (Johnson & Taylor-Holloway, 2006), have also identified a partial down-regualtion in energy intake at subsequent meals following a manipulation in energy intake. Johnson and Taylor-Holloway (2006) demonstrated in 148, 5-12 year old children (53 boys and 68 girls) that following a high energy drink preload (0.63 MJ) and a low energy drink preload (0.13 MJ) the children altered their food intake appropriately at a meal presented 30 minutes later. The mean (±SE) compensation score for the girls was 50.1±9.0%, which significantly decreased with increasing age ($p<0.05$). Johnson and Taylor-Holloway (2006) concluded that 5-12 year old children can partially respond to energy dense cues, although this compensation is incomplete. In studies involving pre-school children, it has been suggested that compensation for energy preloads is likely to occur when a test meal is presented 20-60 minutes following a preload (Wilson, 1999). However, Wilson (1999) also state that when a test meal is presented 90 minutes after energy preload, interference in the compensatory responses occurs, although his has not been extensively explored in older children.

2.5.2 Energy Intake Compensation following Exercise and Exercise-Induced Acute Suppression of Hunger

Typically short-term intervention studies exploring energy intake responses to exercise have not identified a compensatory increase in hunger and food intake, suggesting a loose coupling between energy intake and energy expenditure in the short-term, in young people (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). There is an extensive amount of literature available regarding energy intake and appetite responses to exercise in adults, therefore the reader is referred to review articles by King, Tremblay and Blundell (1997) and Blundell and King (1999) for further information regarding studies involving adults (Reger, Allison & Kurucz, 1984; Thompson, Wolfe & Eikelboom, 1988; Kissileff *et al*., 1990; King, Burley & Blundell, 1994; King & Blundell, 1995; King *et al*., 1996; Imbeault *et al*., 1997; King *et al*., 1997).

The majority of adult-based studies have identified a phenomenen termed 'exercise-induced anorexia', which is a transitory suppression in hunger following exercise (Blundell & King, 1999). It has been identified that this suppression in hunger following exercise is not dependent on the mode of exercise (King & Blundell, 1995), however it is dependent on the intensity and duration of the exercise bout (Thompson, Wolfe & Eikelboom, 1988; King, Burley & Blundell, 1994). This phenomenon has only been identified in males either with high intensity exercise (≥70% $\dot{V}O_{2\,max}$) and/or long duration exercise (~50 minutes), (Thompson, Wolfe & Eikelboon, 1988; King, Burley & Blundell, 1994; King & Blundell, 1995; Broom *et al*., 2007). Even when exercise is of a high intensity (≥70% $\dot{V}O_{2\,max}$) and long duration (~50 minutes), such a transitory suppression in hunger following exercise has not been consistently identified in females (Kissileff *et al*., 1990; King *et al*., 1996). However, King et al. (1996) identified that following high intensity (70% $\dot{V}O_{2\,max}$), long duration (50 minutes) cycling exercise, 13 lean, unrestrained females (22.6±2.3 years) rated foods as more palatable, in comparison to a sedentary condition ($p<0.001$), whilst a significant suppression of hunger was identified during the exercise bout ($p<0.05$).

Similarly to females, a transitory suppression in hunger has not been identified in young girls (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008) and indeed trained adolescent girls (Rumbold & Dodd, 2007), despite a similar lack of energy intake compensation following exercise. Interestingly however, in the first of these studies, Moore et al. (2004) identified that 19 lean girls (10.0±0.6 years) felt they could eat less following a bout of cycling at 75% $\dot{V}O_{2\,max}$ for 38±5 minutes, compared to after a sedentary condition and cycling at 50% $\dot{V}O_{2\,max}$ for 56±7 minutes ($p<0.01$). At a subsequent *ad libitum* lunch however, energy intake did not reflect such subjective appetite sensations. Energy intake at lunch was significantly less following low intensity cycling compared to a sedentary condition, 3.18±1.06 and 3.86±1.02 MJ, respectively ($p<0.05$). These findings have however not been replicated in nine trained adolescent netball players (12.0±0.8 years) (Rumbold & Dodd, 2007). Although the duration of the netball exercise was 47 minutes, the intensity of the exercise was not established, thus the intensity of the exercise may not have been high enough to elicit a change in appetite, as is the case in adults. Consequently, no significant difference in energy intake was identified following the netball exercise and sedentary condition (3.79±1.68 and 3.82±1.59 MJ, respectively). In the most recent of these paediatric studies, appetite responses following exercise in six lean and six overweight girls (11.5±0.4 years), over 5-days were compared (Dodd, Welsman & Armstrong, 2008). Following cycling at 75% $\dot{V}O_{2\,max}$ for a duration long enough to elicit 0.75 MJ energy expenditure, over two consecutive days, there was no change in subjective hunger, fullness or prospective food consumption in the lean girls (Dodd, Welsman & Armstrong, 2008). The overweight girls however felt significantly more hungry ($p<0.001$) and less full ($p<0.001$) immediately following the cycling exercise compared to immediately before (Dodd, Welsman & Armstrong, 2008). Similarly to the study of Moore et al. (2004) such subjective appetite sensations were not reflected in energy intake responses. Total daily energy intake or total 5-day energy intake across or between conditions, for lean and overweight girls were not significantly different. Unlike in adults, there is no consensus with regards to appetite responses following exercise in paediatric groups.

In terms of energy intake responses to exercise, however the consenus from short-term intervention studies in young girls and trained adolescent girls, indicates that a period of 7 days or less may not be long enough, for any compensatory increase in energy intake to occur (Blundell *et al*., 2003). The reader is refered to several reviews for an outline of the medium and long-term intervention studies investigating appetite and energy intake following exercise (Blundell & King, 1999; Blundell *et al*., 2003). Some studies have identified a compensatory increase in energy intake following exercise, which are outlined in the following section.

2.5.3 Partial and Full Energy Intake Compensation following Exercise

In one of the most classically cited cross-sectional studies, Edholm et al. (1955) monitored free-living energy intake and energy expenditure of 12 male cadets (19.0±5.6 years old) over 2-weeks of army training. Cafeteria food was provided and was weighed before and after eating, whilst energy expenditure was assessed using metabolic determinants. Edholm et al. (1955) identified that energy intake and energy expenditure were matched over 7-days, equating to 14.34 and 14.17 MJ, respectively. It was also established that energy expenditure on 1 day was matched by energy intake 2 days later. Adolescent gymnasts (mean age 16 years) (Parizkova & Poupa, 1963) involved in systematic training for sport, have also demonstrated compensatory increases in energy intake in response to varying levels of exercise or training. The reader is referred to several reviews with regards to cross-sectional energy intake reponses to exercise (Blundell & King, 1999; Blundell *et al*., 2003).

The majority of adult-based short-term intervention studies, that have identified partial or full energy intake compensation for exercise-induced energy expenditure and an elevation in subjective hunger, have had certain characteristics. These have included i) trained participants performing representative sport-specific exercise (Verger, Lanteaume & Louis-Sylvestre, 1992; Verger *et al*., 1992), similar to that of Edholm et al. (1955) and Parizkova and Poupa (1963); ii) exercise has lasted for a fairly long duration (120 minutes) (Verger, Lanteaume & Louis-Sylvestre, 1992, 1994; Erdmann *et al*., 2007); iii) studies have been conducted in free-living environments in adult males and females (Stubbs *et al*., 2002a,b).

With regards to the imposition of sport-specific exercise, Verger, Lanteaume and Louis-Sylvestre (1992) conducted one of the first studies in this domain. Sixty minutes and 120 minutes following 2-hours of submaximal athletic activity, five trained women and eight trained males increased their energy intake at an *ad libitum* meal by 1.97 MJ, compared to a sedentary condition. When the meal was presented immediately after the exercise bout no change in energy intake was found. Furthermore, compared to immediately after the exercise condition, carbohydrate as a percentage of energy intake increased significantly when an ad libitum meal was presented 60 minutes post-exercise

($p<0.05$) and 120 minutes post-exercise ($p<0.01$) (Verger, Lanteaume & Louis-Sylvestre, 1992). The percentage of calories consumed as protein decreased with increasing time ($p<0.01$). Subjective hunger was also elevated following the exercise bout compared to the corresponding time in the sedentary condition ($p<0.01$). However, using the same methodology, a similar energy intake response to submaximal exercise was identified in sedentary males (Verger, Lanteaume & Louis-Sylvestre, 1994), thus suggesting that training status may not be the only influencial variable. Unfortunately, the two cited studies (Verger, Lanteaume & Louis-Sylvestre, 1992; 1994) had several shortcomings. Firstly, there were no calorimetry measurements of the exercise sessions and therefore no indication of the exercise intensity of the sessions or the energy expenditure induced by the exercise (King, Tremblay & Blundell, 1997). Furthermore, Verger, Lanteaume and Louis-Sylvestre (1994) also used a between-groups design, thus the integrity of these findings can be questioned since the same participants did not participate in both conditions.

Interestingly however, Erdmann et al. (2007), using a more robust methodology also identified an increase in energy intake following exercise. In three untrained females and four untrained males, energy intake was significantly higher after long duration cycling exercise (50W; 120 minutes) when compared to a sedentary condition ($p=0.049$), a short duration cycling condition (50W; 30 minutes) ($p=0.001$) and a moderate duration cycling condition (50W; 60 minutes) ($p=0.001$), 3.26±0.51, 2.36±0.14, 2.00±0.41 and 2.39±0.50 MJ, respectively. Taken together, the findings of Verger, Lanteaume and Louis-Sylvestre (1992; 1994) and Erdmann et al. (2007) suggest that energy intake compensation following exercise may occur if exercise is of a long duration and that a compensatory response is not necessarily dependent on training status.

Another plausible suggestion as to why energy intake compensation following exercise occurs is the method by which food is made available to particpants following exercise trials. Studies that have not identified a compensatory increase in energy intake following exercise, have typically presented participants with an *ad libitum* buffet meal at various time points after exercise has been performed. However, in the first study to identify a compensatory increase in energy intake following exercise, food was made available to the participants at their own discretion throughout the trial, in an attempt to replicate a free-living environment (Durrant, Royston & Wolch, 1982). Findings from this study suggest that when food is available for consumption at the discretion of participants, who do not necessarily have to be trained with regards to the exercise intervention, an increase in energy intake following exercise is likely to be identified. Durrant, Royston and Wolch (1982) identified that an increase in energy intake was due to an increase in eating frequency, not an increase in absolute energy intake at individual eating occasions. Consequently, an elevation in energy intake following exercise may not be identified when participants are provided with set meals, as in all of the short-term intervention studies, which identified a lack of change in energy intake in response to exercise. It seems that the imposition of free-living conditions in exercise and appetite studies is advantageous to identify a compensatory increase in energy intake following exercise.

This argument is further supported by a more recent study involving 6 lean untrained women mean (±SE) 23.0±0.6 years, where partial energy intake compensation following exercise was identified when research was conducted in a free-living environment (Stubbs *et al*., 2002b). Over 7 days participants completed either a sedentary condition, a medium exercise condition (2 x 40 minute cycling bouts per day / 1.9 $MJ \cdot d^{-1}$) and a high exercise condition (3 x 40 minute cycling bouts per day / 3.4 $MJ \cdot d^{-1}$). Free-living food intake was recorded by the participants using self-reported weighed food diaries. Subjective appetite, hunger and satiety were assessed using VAS at hourly intervals on each day and an end of day questionnaire completed to further explore these parameters along with mood. Energy intake was significantly increased by 1.1 $MJ \cdot d^{-1}$ in the high exercise condition compared to the sedentary condition. This increase in energy intake only partially compensated for the elevated energy expenditure (33%). Similarily to Durrant, Royston and Wolch (1982) carbohydrate intake was significantly higher in the high exercise condition compared to the sedentary condition, 4.9 versus 5.4 MJ, respectively (p=0.037). Interestingly, the elevation in energy intake corresponded to subjective hunger, since the end of day questionnaire identified that participants were significantly more hungry during the medium and high exercise conditions compared to the sedentary condition (p=0.008).

In summary, partial energy intake compensation for exercise-induced energy expenditure has been identified in females when studies have been conducted in a free-living environment (Durrant, Royston & Wolch, 1982; Stubbs *et al*., 2002a,b). Despite the free-living conditions of the research (Durrant, Royston & Wolch, 1982; Stubbs *et al*., 2002a,b), it seems 3-days (Durrant, Royston & Wolch, 1982) and 7-days (Stubbs *et al*., 2002a,b), do not appear to be long enough periods for full energy intake compensation to occur, following a period of elevated energy expenditure (Blundell *et al*., 2003).

Typically and as discussed previously (see section 2.2.2), subjective appetite parameters such as hunger, prospective food consumption and fullness, using VAS are normally measured alongside estimations of energy intake, in exercise and appetite research studies. There is however some inconsistencies in appetite responses following exercise with regards to paediatric populations (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). Ideally therefore, the measurement of acylated ghrelin concentrations in paediatric populations following exercise, would provide an objective measure of appetite in conjunction with subjective measures of appetite, to explore the inconsistencies identified using VAS.

2.5.4 Short-Term Ghrelin Responses to Exercise

As discussed previously, VAS (see section 2.2.2) are a subjective means of assessing appetite, more specifically hunger, prospective food consumption and fullness. It seems from the short-term intervention studies discussed, a measure of subjective hunger with regards to exercise is informative and useful, although a concurrent, objective measure of hunger in the form of hormone analysis (ghrelin) would provide additional vital information. This procedure is costly however and the preparation of the assay for the analysis of acylated ghrelin is time consuming.

All the available research concerning ghrelin responses to exercise have so far only been conducted in adult males and females, with no studies exploring ghrelin responses to exercise in paediatric populations. The studies, which have been conducted, have explored the release of ghrelin in response to exercise in active/trained and inactive/untrained individuals. The findings from studies conducted so far are equivocal, with some identifying no change in ghrelin following exercise, an increase in ghrelin following exercise or a decrease in ghrelin following exercise. In addition the majority of studies have explored total ghrelin concentrations following exercise as opposed to acylated ghrelin (the importance of this will be discussed later in this section).

Studies that have explored plasma total ghrelin concentrations in relation to exercise in untrained adults, have found that exercise had no influence on total ghrelin concentrations (Kallio, Pesonen & Karvonen, 2001; Dall *et al*., 2002; Schmidt, Maier & Schaller, 2004; Martins *et al*., 2007). Only two of these studies have involved healthy participants (Schmidt, Maier & Schaller, 2004; Martins *et al*., 2007). Martins et al. (2007) recruited six sedentary males and six sedentary females (25.9±4.6 years old) in order to explore subjective appetite, energy intake and total ghrelin concentrations following intermittent cycling exercise at 65% of age estimated maximum heart rate for 60 minutes or a 60 minute rest condition. Prior to each trial the participants were provided with a standardised breakfast. Two baseline blood samples were collected via cannulation followed by additional blood samples at regular intervals for a further 3 hours. Visual analogue scales assessing subjective hunger, prospective food consumption and fullness were administered simultaneously at each blood collection. One hour following the exercise or rest trial an *ad libitum* buffet meal was provided. Martins et al. (2007) found that total ghrelin levels were not influenced by exercise, however interestingly subjective hunger was significantly lower (p=0.004) during exercise and immediately after, although this was short lived. Absolute energy intake at the buffet meal was significantly higher following exercise compared to the control trial 3.8±1.5 versus 3.2±1.1 MJ, respectively (p<0.05), whilst energy intake relative to the energy expended during each trial was significantly lower in the exercise trial compared to the control trial, 1.8±1.3 versus 2.4±0.9 MJ, respectively (p=0.038). Given the evidence which suggests that males and females respond differently to exercise with regards to subjective appetite (King *et al*., 1996), it would have been more appropriate for Martins et al. (2007) to have analysed these results separately in terms of gender.

In contrast to these findings with respect to total ghrelin concentrations (Schmidt, Maier & Schaller, 2004; Martins *et al*., 2007), Vestergaard et al. (2007) found a decrease in total ghrelin concentrations following running exercise to exhaustion, in 20 sedentary males and 12 sedentary females (24.6±0.6 years old). Similarily, in contrast to the findings of Vestergaard et al. (2007), Erdmann et al. (2007) identified an increase in total ghrelin concentrations following low intensity exercise in seven sedentary individuals (two males, five females) (24.4±0.6 years).

Interestingly, however, the majority of studies (all in adults) which have identified a change in total ghrelin levels with exercise have mainly involved trained participants who completed exercise interventions which were representative of sporting activities which they systematically trained for, as opposed to only being classified as habitually active (Christ *et al*., 2006; Jürimäe *et al*., 2007b; Jürimäe, Jürimäe & Purge, 2007; Vestergaard *et al*., 2007). The three latter studies will be discussed in detail, since the earliest of these studies (Christ *et al*., 2006) was concerned with manipulating diet with regards to fat intake prior to a 3 hour cycling intervention. In the first of these studies, Jürimäe et al. (2007b) recruited nine elite male rowers (20.1±3.7 years old) to explore total ghrelin responses to sculling exercise. The participants were asked to complete a 30 minute bout of constant load sculling exercise on water after having consumed a standardised breakfast 2 hours prior. The participants were randomised to two exercise trials, one which involved rowing below their individual anaerobic threshold and one which involved rowing above their individual anaerobic threshold, with at least 2-4 days rest between trials. Mean (±SD) anaerobic threshold equated to 79.3±6.2% $\dot{V}O_{2\,max}$. Blood samples were collected immediately before and after exercise and 30 minutes post exercise in order to explore total ghrelin concentrations. Total ghrelin concentrations 30 minutes post exercising above anaerobic threshold were different to total ghrelin concentrations immediately pre-exercise (855±169 and 802±181 pg·ml^{-1}, respectively). Although this difference was not significant it was approaching significance (p=0.051). Jürimäe et al. (2007b) concluded that the difference did not reach significance since the exercise bout may not have induced a large enough negative energy balance. Indeed, Ravussin et al. (2001) states that ghrelin may be a sensor of negative energy balance.

Consequently, researchers from the same group (Jürimäe, Jürimäe & Purge, 2007) adapted the above study, with the intention of elevating the energy cost of the exercise bout further, which they believed was necessary in order to find a significant change in total ghrelin concentrations. Following a standardised breakfast, eight elite male rowers (21.3±2.8 years old) each completed a maximal 6000 m time trial on a rowing ergometer, which took on average 19.52±0.27 minutes. Exercise intensity equated to approximately 81% $\dot{V}O_{2\,max}$, with energy expenditure estimated at 1.7 MJ. Blood samples were collected at the same time points as above, immediately pre and post exercise and 30 minutes post exercise to explore total ghrelin concentrations. Jürimäe, Jürimäe and Purge (2007) identified that ghrelin levels were significantly elevated by 24.4% immediately after the exercise in comparison to immediately before (p<0.05). Jürimäe, Jürimäe and Purge (2007) concluded that total ghrelin levels increased following maximal rowing exercise in elite male rowers and suggested that total ghrelin may be a peripheral marker of a negative energy balance and that during exercise, signals to initiate metabolic reactions to meet energy needs originate from this hormone (Jürimäe, Jürimäe & Purge, 2007).

A limitation of the available published studies exploring ghrelin responses to exercise is that total ghrelin has typically been measured as opposed to acylated ghrelin (Broom *et al*., 2007) and therefore may explain in some instances why changes in subjective appetite and energy intake have occurred without a change in total ghrelin concentrations. Acylated ghrelin is the most active form of ghrelin, although the majority of circulating ghrelin (80-90%) is non-acylated (Hosoda *et al*., 2004; Ghigo *et al*., 2005). Non-acylated ghrelin is unable to bind and activate the growth hormone-secretagoue receptor and is therefore devoid of any affects on the endocrine axis (Ghigo *et al*., 2005), whereas acylated ghrelin has the ability to bind and therefore cross the blood-brain barrier (Kojima *et al*., 1999; Murphy & Bloom, 2006). Acylated ghrelin possess pituitaric and pancreatic endocrine activities and consequently is considered to be important for appetite regulation (Broglio *et al*., 2003) and is therefore a more appropriate objective measure of appetite or more specifically hunger. However it has not been extensively studied, as it is less stable than total ghrelin (Hosoda *et al*., 2004) and therefore very difficult and time consuming to collect and process (Broom *et al*., 2007).

Secondly, it has also been the case that studies investigating the relationship between total ghrelin and exercise have either standardised diets prior to trials being completed (Kallio, Pesonen & Karvonen, 2001; Dall *et al*., 2002; Christ *et al*., 2006; Broom *et al*., 2007; Erdmann *et al*., 2007; Jürimäe *et al*., 2007b; Jürimäe, Jürimäe & Purge, 2007; Martins *et al*., 2007) or imposed an overnight fast (Kramer *et al*., 2004; Schmidt, Maier & Schaller, 2004; *et al*., 2007; Burns *et al*., 2007; Vestergaard *et al*., 2007). Firstly, with regards to acute exercise-induced responses of ghrelin there is no consensus on the effect of either caloric intake or restriction (Jürimäe *et al*., 2007b; Jürimäe, Jürimäe & Purge, 2007). However, 1 hour after a meal total ghrelin concentrations are known to reach a nadir and that after a meal changes in ghrelin concentrations are smaller compared to after an overnight fast in healthy adults (Cummings *et al*., 2001). Despite this however, it has also been stated that an elevation in total ghrelin due to exercise maybe masked if participants fast overnight prior to trials (Kraemer *et al*., 2004). Secondly, both these conditions are not representative of free-living situations, consequently there is a relative dearth of information concerning acylated ghrelin responses to exercise following a period of free-living food intake.

Broom et al. (2007) conducted one of the first studies to explore acylated ghrelin and subjective hunger responses following running exercise in nine habitually active males (21.1±0.7 years old). The participants were involved in sports such as soccer, rugby, tennis and hockey. The participants completed an exercise trial and a control trial, which were separated by at least 7 days. Following an overnight fast, each trial began at 08:00 and lasted 9 hours, whilst the participants completed an exercise condition which involved 60 minutes of treadmill running at 75% $\dot{V}O_{2\,max}$, or a control condition which involved completing resting activities. Two hours after completion of each condition a test meal was provided of a set macronutrient content of 38% carbohydrate, 10% protein and 52% fat, which the participants had 15 minutes to consume. Venous blood samples via cannulation were collected at 08:00, 08:30, 09:00, 11:00, 12:00 and 17:00 to explore acylated ghrelin, whilst Lickert scales assessing subjective hunger were administered at baseline, every 30 minutes thereafter for 5 hours and every hour thereafter until the end of each trial. Results demonstrated that over the first 3 hours of the trial mean (±SE) area under the curve acylated ghrelin levels were significantly lower (p<0.05) in the exercise trial compared to the control trial (317±135 versus 510±186 pg·ml^{-1}, respectively). This was also the case for ghrelin concentrations over the 9 hour duration in the exercise trial compared to the control trial (917±342 versus 1,401±521 pg·ml^{-1} (p<0.05). Subjective hunger was also significantly decreased over 3 hours in the exercise trial compared to the control trial (p=0.013). Over the first 3 hours of the exercise trial a significant correlation between hunger and acylated ghrelin was also identified (r=0.699, p=0.036). At individual time points, correlations between hunger ratings and acylated ghrelin were identified in control trial at 08:00. 08:30. 09:00 and 17:00 and in the exercise trial at 09:00 (r=0.715 – r=0.894, p<0.05). Broom et al. (2007) therefore concluded that during an acute bout of treadmill running plasma acylated ghrelin levels are reduced, providing an explanation for subjective hunger being suppressed, otherwise referred to as 'exercise-induced anorexia' (Blundell & King, 1999) in males during and immediately after exercise.

Taken together, studies exploring ghrelin responses to exercise in adults are equivocal, with the majority exploring total ghrelin concentrations and one study to date exploring acylated ghrelin concentrations (Broom *et al*., 2007). The above work needs to be extended using trained female groups and specifically adolescent girls (trained and untrained), especially since subjective appetite responses following exercise in these sample populations have been found to vary compared to those of adults.

2.5.5 Summary of the Short-Term Energy Intake, Macronutrient Selection, Subjective Appetite and Ghrelin Concentrations following Exercise

All of the intervention studies involving paediatric groups with regards to energy intake and appetite following exercise, have demonstrated that an increase in energy expenditure is not necessarily immediately followed by an up regulation in energy intake (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). However free-living exercise and appetite studies using paediatric populations are lacking. In addition the subjective appetite data from the studies by Moore et al. (2004), Rumbold and Dodd (2007) and Dodd, Welsman and Armstrong (2008) are inconsistent, demonstrating the need for a more objective measure of appetite such as acylated ghrelin concentration changes. There is also a dearth of information regarding trained individuals and the imposition of sport-specific exercise, with only one paediatric study to date having imposed sport-specific exercise in an intervention study (unpublished) using trained participants (Rumbold & Dodd, 2007).

Therefore, the overall aim of this thesis was to establish whether free-living energy intake, subjective appetite and objective appetite (acylated ghrelin concentrations) would be influenced by a bout of netball specific exercise in female adolescent netball players. See section 1.1 for the detailed aims of this thesis.

CHAPTER 3

Assessment of Physical and Physiological Parameters in General and Sport-Specific Tests for Adolescent Netball Players aged 13-15 years

3.1 Introduction

Netball fitness tests have been developed for use with adults but not young people (Gasston & Simpson, 2004), therefore prior to using such a test to assess appetite and food intake responses to sport-specific exercise, the agreement with traditional laboratory based ergometer tests needs to be investigated.

In the laboratory there are a variety of methods which can be used to assess the $\dot{V}O_{2\ peak}$ of children and adolescents and therefore used to predict subsequent exercise intensity, with the cycle ergometer and the treadmill being the most frequently used (Fawkner & Armstrong, 2007). When working with children and adolescents, laboratory based incremental treadmill tests are well established for assessing $\dot{V}O_{2\ peak}$ and are typically the method of choice (Sheehan, Rowland & Burke, 1987). However, when direct laboratory assessment is unavailable, a field-based fitness test known as the multi-stage fitness test (Leger & Lambert, 1982), enables the $\dot{V}O_{2\ peak}$ of young people to be predicted. The All England Netball Association use the multi-stage fitness test to predict aerobic fitness for players of all ages, although the only fitness element this measures is peak running speed (National Coaching Foundation, 1995) and not other qualities, which are important to netball performance. The suitability of the multi-stage fitness test to assess young people's aerobic fitness has also been questioned since the predictions of aerobic fitness are based on adult-specific equations.

The importance of sport-specific testing of aerobic fitness has previously been demonstrated by St Clair Gibson et al. (1998). This group assessed the $\dot{V}O_{2\ max}$ of 10 long distance runners and 10 squash players using an incremental treadmill protocol and correlated these values to scores predicted from the multi-stage fitness test. For the runners the scores were more highly correlated ($r = 0.71$) compared to those of the squash players ($r = 0.61$). St Clair Gibson et al. (1998) concluded that when predicting $\dot{V}O_{2\ max}$ from the multi-stage fitness test there are likely to be sport-specific differences. The general consensus is that a more representative value for $\dot{V}O_{2\ peak}$ is provided if the activities simulate the movement patterns completed in training and competition (Fawkner & Armstrong, 2007). In terms of exercise and appetite research, a representative value for

$\dot{V}O_{2\ peak}$ in active young people during preliminary testing procedures enables the exercise intensity of subsequent exercise intervention periods to be more accurately assessed. Indeed, this is especially pertinent when considering the effect exercise intensity has on subjective appetite, with 'exercise-induced anorexia' potentially occurring at 70% $\dot{V}O_{2\ max}$ (King, Burley & Blundell, 1994).

Consequently, the appropriateness of non-specific tests such as incremental treadmill tests and the Multi-Stage Fitness Test for assessing netball specific aerobic power is problematic, due to the lack of specificity (Thissen-Milder & Mayhew, 1991). A netball specific fitness test has been developed by Gasston and Simpson (2004) incorporating movement patterns which simulate those of netball activities, such as walking forwards and backwards, jogging forwards and backwards, running forwards and backwards, turning, jumping, lunging, sidestepping, foot-specific agility and a choice reaction task. Thus, unlike incremental treadmill tests and the multi-stage fitness test, which only involve forward running, a netball-specific test has the potential to provide a representative assessment of netball-specific aerobic power. Originally this test was developed for use with adults, however for the purpose of the studies in chapters five and six of this thesis, modifications have been made to account for the age of the participants (13-15 years old). Following initial pilot testing, modifications to the test involved reducing the demand of the stages. In schools, players typically train and play on netball courts with smaller dimensions, therefore the protocol was adapted to replicate these dimensions as closely as possible. The test was also converted into an incremental field-based netball fitness test, to directly assess aerobic power, providing a numerical value for $\dot{V}O_{2\ peak}$ as opposed to a level and stage score as was the case in the original version.

Therefore, the aims of this study were to develop a modified field-based netball fitness test for use with young people and to explore how this test compared to a standard laboratory based incremental treadmill test and a field-based multi-stage fitness test.

3.2 Methods

3.2.1 Design

Using a within-subject, randomised cross over design, the study compared $\dot{V}O_{2\ peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) values of trained adolescent female participants derived from a field-based netball fitness test, an incremental treadmill test and a field based multi-stage fitness test.

3.2.2 Participants

Participants aged 13-15 years old who were part of the school year 7, 8 and 9 netball squads at a local secondary school were invited to take part in the study. From those that responded, inclusion was based on four criteria. Firstly, only girls with a healthy BMI were able to participate in the study. This was determined using the age and gender-specific classification of Cole et al. (2000) for the United Kingdom, where BMI scores which fell between the 9th centile and the 91st centile were accepted as an indicator of a healthy BMI. Secondly, the girls were required to be actively involved with netball training sessions or matches two to three times per week, in order to be classified as trained with regards to netball. This was confirmed by liaising with the physical education staff at the school and the netball coaches from the clubs the participants attended, along with scrutiny of weekly attendance registers. Thirdly, a medical questionnaire was administered prior to the study, to identify any injuries which potentially would have prevented completion of the three tests. Finally, any participants who were unable to attend any of the testing dates were also excluded.

The study was approved by the School of Psychology and Sport Sciences Research Ethics Committee at the University of Northumbria. The participants and their parents or guardians were provided with information sheets detailing the procedures and requirements of the study. They were informed that the study was based on assessing fitness levels in adolescent netball players using a netball fitness test. They were not told that these results would be directly compared to the results obtained from the incremental treadmill test and the multi-stage fitness test.

Twelve participants commenced the study and subsequently written informed consent was obtained from both the participants and parents or guardians prior to data collection (see appendix A for an example of the consent forms). From the 12 girls who participated there was a wide range of player position [Goal Keeper x 2, Goal Defence x 2, Wing Defence x 1, Centre x 2, Wing Attack x 1, Goal Attack x 2, Goal Shooter x 2]. On separate days, prior to testing, all the participants were habituated to the full test procedures for the netball fitness test, the incremental treadmill test and the multi-stage fitness test.

3.2.3 Body Composition Assessment

During a preliminary testing week, stature and seated height were measured to the nearest 0.01 m using a Harpenden Portable Stadiometer (Holtain Limited, Pembs, UK) with participants wearing light weight sports kit and no trainers. Body mass was measured to the nearest 0.1 kg using Avery Balance scales (Avery Berkel Ltd, West Midlands, UK). BMI was derived from these parameters, calculated as body mass (kg)/stature (m^2). Body density was assessed by air displacement plethysmography using a Bod Pod (Life Measurement Instruments, Concord, CA, USA), and converted to percentage body fat using the age and gender-specific equations of Lohman (1989). The participants abstained from exercise and food consumption for 2 hours prior to entering the Bod Pod. Two same gender technicians were present whilst each participant was in the Bod Pod.

3.2.4 Maturity Offset

Maturation status was calculated from the anthropometric measurements (stature, seated height, mass, leg length) and chronological age using the gender-specific regression equations developed by Mirwald et al. (2002). This provided a value for maturity offset which gave an indication of the time lag (in years) before or after the predicted peak height velocity of each participant.

3.2.5 Study Protocol

Following the preliminary testing week, each girl completed each of the fitness tests (netball fitness test, incremental treadmill test and the multi-stage fitness test), in a randomised order, over a 3 week testing period, with a 'wash out' period of at least 7 days between each test (table 3.1).

Table 3.1. Outline of study protocol.

	Week 1	**Week 2**	**Week 3**
Group 1 (n=4)	Multi-stage fitness test	Netball fitness test	Incremental treadmill test
Group 2 (n=4)	Incremental treadmill test	Multi-stage fitness test	Netball fitness test
Group 3 (n=4)	Netball fitness test	Incremental treadmill test	Multi-stage fitness test

Each test was conducted on the same week day at approximately the same time, for each participant, therefore the girls were allocated to groups dependent on which day they were available to participate in the study. All tests were conducted on school premises. Similar environmental conditions were present during all three tests (temperature 17.6±0.3^{o}C, relative humidity 53±1%, barometric pressure 1016±3 mBar) and the girls were asked to wear similar clothing and footwear for each test. On each of the test days the girls were told to refrain from other exercise and also refrain from eating for 2 hours prior to each test. Researchers liaised with the physical education staff at the school to ensure this.

Four of the participants from group three, also performed a second trial of the netball fitness test (Gasston & Simpson, 2004) 14 days after the first to assess the reproducibility of scores for all parameters ($\dot{V}O_{2\ peak}$, heart rate at $\dot{V}O_{2\ peak}$, respiratory exchange ratio at $\dot{V}O_{2\ peak}$ and time to reach $\dot{V}O_{2\ peak}$). These trials were conducted on the same week day for each participant, at approximately the same time their previous test was performed and the participants were told to wear the same footwear and clothing.

3.2.6 Peak Oxygen Uptake

During the incremental treadmill test and the netball fitness test, breath-by-breath oxygen uptake was monitored using a facemask, attached via breathing tubes to a Metamax 3B portable gas analyser (Cortex, Biophysik, Leipzig, Germany). Prior to each test this equipment was calibrated using the guidelines provided by the manufacturers. An individual's $\dot{V}O_{2\ peak}$ was identified as the highest oxygen uptake recorded during any 60 second interval (St Clair Gibson *et al.*, 1998). The criteria used to determine when $\dot{V}O_{2\ peak}$ had been reached were based on those proposed by Armstrong and Welsman (2001). These included voluntary exhaustion, despite continued verbal encouragement (non-standardised), subjective endpoints such as facial flushing, sweating, hyperpnoea and an unsteady gait, and either heart rate levelling off or a respiratory exchange ratio ≥ 1.00.

3.2.7 Heart Rate

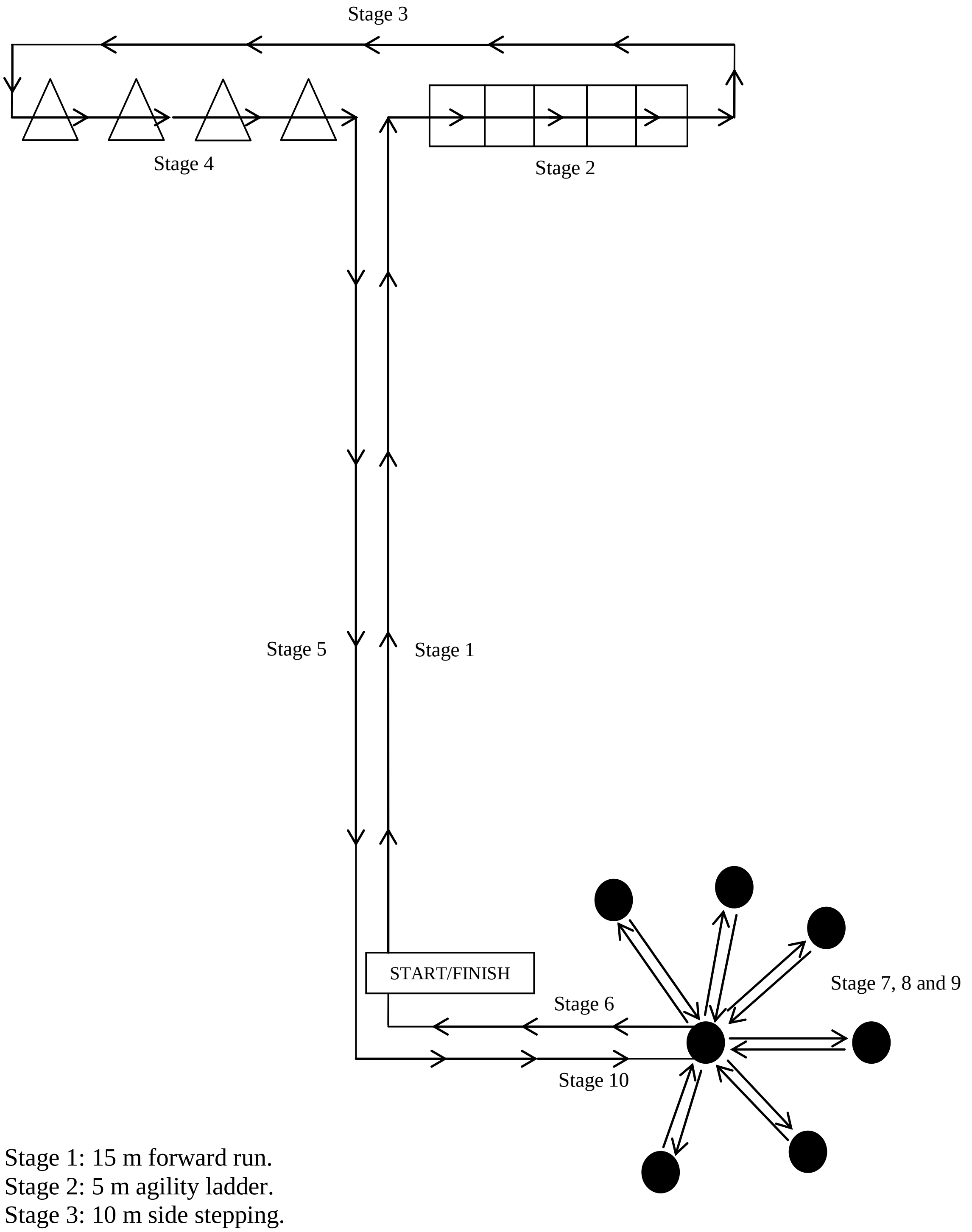
During all three tests heart rate was monitored by portable heart rate telemetry (Polar RS-400 and Polar S610i). Each participant was asked to wear a transmitter strap around their chest and a watch-like receiver on their wrist. Heart rate was averaged over 60 second epochs. In all three tests, heart rate at $\dot{V}O_{2\ peak}$ was denoted to be the heart rate value, which corresponded with the $\dot{V}O_{2\ peak}$ value.

3.2.8 Ratings of Perceived Exertion

The Pictorial Children's Effort Rating Table was administered to assess ratings of perceived exertion at the beginning and end of each test and at the end of each respective stage in all three tests. The scale ranged from 1 to 10, 1 depicting the exercise as being *very, very easy* and 10 as being *so hard I'm going to stop.* This scale has been found to successfully monitor paediatric effort perception in adolescents aged 11-15 years whilst they completed various exercise tasks of differing physiological demands (Yelling, Lamb & Swaine, 2002).

3.2.9 Netball Test

The progressive netball fitness test began with a 3 minute warm up at level 1. The test consisted of 10 levels which each lasted 3 minutes. Each level comprised of ten stages illustrated in figure 3.1. The movements were identified from a needs analysis conducted by Gasston and Simpson (2004) and therefore all movements were specific to those involved when playing netball (walking forwards and backwards, jogging forwards and backwards, running forwards and backwards, turning, jumping, lunging, sidestepping, foot-specific agility and a choice reaction task). The netball fitness test is represented schematically in figure 3.1.



Stage 1: 15 m forward run.
Stage 2: 5 m agility ladder.
Stage 3: 10 m side stepping.
Stage 4: two footed jumps over four 30 cm hurdles, covering 5 m.
Stage 5: 15 m backward run.
Stage 6: complete three 5m runs between cones.
Stage 7, 8 and 9: lunge towards one coloured cone twice, each 3 m from centre cone (repeat using a different coloured cone for stages 8 and 9).
Stage 10: complete three 5 m runs between cones.

Figure 3.1. Organisation of the netball fitness test.

Speed of progression through the stages at each level was regulated using an audio CD which emitted a sound signal indicating to the participants when to progress to the next stage. The participants were told to stay in time with the sound signal, arriving at the beginning of the next stage as a signal was given, not before or after. The durations for the stages in each level are presented in table 3.2.

Table 3.2. Linear adjusted stage durations for each level.

Level	Duration of each stages (seconds)
1	10
2	9.3
3	8.6
4	7.9
5	7.2
6	6.5
7	5.8
8	5.1
9	4.4
10	3.7
11	3

In netball the majority of work periods are under 10 seconds for all positions (Otago, 1983), hence the duration of the stages in level 1. Three second durations for the stages in level 11 were also identified by Otago (1983) who states that there are a high number of occurrences of work periods for all positions, which last less than 4 seconds. From previous pilot tests, this was also the most appropriate work period to use for level 11 as the participants were able to stay in time with the sound signal, which in itself was demanding. In order to make the decrements in time for the duration of each stage in each level linear, the duration of each stage for levels 2-10 were derived from a regression equation. This was calculated using the stage durations of level 1 and level 11, thus $y = -0.7x + 10.7$, where x was the level and therefore y was the duration of each stage within that level.

The girls were told to complete as many circuits of the test as possible. The test was terminated if the girls could not continue despite verbal encouragement and if the subjective and objective $\dot{V}O_{2\ peak}$ criteria proposed by Armstrong and Welsman (2001) had been attained.

3.2.10 Incremental Treadmill Test

The progressive incremental treadmill test began with a 3 minute warm up at 6 km·h^{-1} on a motorised treadmill (Life Fitness, UK). For the first 3 minutes of the test the speed was increased to 8 km·h^{-1} and then increased by 1 km·h^{-1} every 3 minutes until 10 km·h^{-1} was reached. If $\dot{V}O_{2\ peak}$ had not been achieved the speed was kept constant and the gradient increased by 2.5% per minute until the participants could not continue despite verbal encouragement and if the subjective and objective $\dot{V}O_{2\ peak}$ criteria proposed by Armstrong and Welsman (2001) had been attained.

3.2.11 Multi-Stage Fitness Test

This standard field based fitness test consisted of 23 levels each lasting 1 minute. Each level comprised of 20 m shuttle runs, the first level having a starting of speed 8.5 km·h^{-1}, which was increased by 0.5 km·h^{-1} at each level (Leger & Lambert, 1982). An audio CD which emitted a sound signal indicated when the participants needed to run from one end of the 20 m length to the other. This enabled the running speed to be regulated. All the girls were told to complete as many shuttle runs as possible. The test was terminated if the girls could not continue despite verbal encouragement and if the subjective and objective $\dot{V}O_{2\ peak}$ criteria proposed by Armstrong and Welsman (2001) had been attained.

3.2.12 Statistical Analysis

The statistical package SPSS-PC v 15.0 (SPSS Inc., Chicago, IL) was used for all data analyses (ANOVA outputs for all studies are provided in appendix B). Mean and standard deviation were calculated for each of the parameters sampled. Prior to analysis all data was assessed for normality using the Shapiro-Wilks' test. For all three fitness tests, $\dot{V}O_{2\ peak}$, heart rate at $\dot{V}O_{2\ peak}$, Pictorial Children's Effort Rating Table at $\dot{V}O_{2\ peak}$ and time to reach $\dot{V}O_{2\ peak}$ were analysed using separate one way repeated measure ANOVA. Since the respiratory exchange ratio was not sampled during the multi-stage fitness test, a one way repeated measures ANOVA (within-design) was conducted to compare the values obtained during the incremental treadmill test and the netball fitness test only. Tukey tests were used for post-hoc analyses to determine the nature of any effects if the *F*-ratios were significant. To determine the size of any effects, Cohen's d effect size was calculated for significant differences, assuming a small effect size = ≤ 0.2, a moderate effect ~ 0.5, and ≥ 0.8 = large effect (Cohen, 1988; 1992). To determine if sphericity had been violated a Mauchly's sphericity test was performed. If a violation of sphericity had occurred the Greenhouse-Geisser correction was applied.

Coefficients of variation (%) for the netball fitness test, test-retest reproducibility for $\dot{V}O_{2\ peak}$ (ml·kg^{-1}·min^{-1}), heart rate at $\dot{V}O_{2\ peak}$ (b·min^{-1}), respiratory exchange ratio at $\dot{V}O_{2\ peak}$ and time to reach $\dot{V}O_{2\ peak}$ (min:sec) were calculated.

The significance level was set at $p<0.05$ for all analyses.

3.3 Results

The age and physical characteristics (mean ± SD) of the participants were: age 14.1±0.8 years; height 1.61±0.08 m; weight 54.1±8.9 kg; BMI 20.5±2.4 kg/m^2; and body fat 23.2±4.0%. The maturity offset was 1.8±0.8 years from peak height velocity, indicating that all the girls had reached their predicted peak height velocity (positive maturity offset) and were thus of a similar maturation status.

3.3.1 Peak Oxygen Uptake

When the mean ± SD $\dot{V}O_{2\ peak}$ values obtained from the three tests were compared, there was a main effect of test type [$F(2,22)=8.947$, $p=0.001$]. Post-hoc Tukey tests revealed that $\dot{V}O_{2\ peak}$ values obtained from the incremental treadmill test were significantly higher than those obtained from the netball fitness test, 44.6±3.6 versus 40.5±4.3 ml·kg^{-1}·min^{-1} ($p<0.01$, effect size=1.08), respectively. Values obtained from the multi-stage fitness test were also significantly higher than those obtained from the netball fitness test, 44.5±3.6 versus 40.5±4.3 ml·kg^{-1}·min^{-1} ($p<0.01$, effect size=1.05), respectively.

Figure 3.2 indicates $\dot{V}O_{2\ peak}$ values which were obtained from the netball fitness test compared to the incremental treadmill test and the multi-stage fitness test.

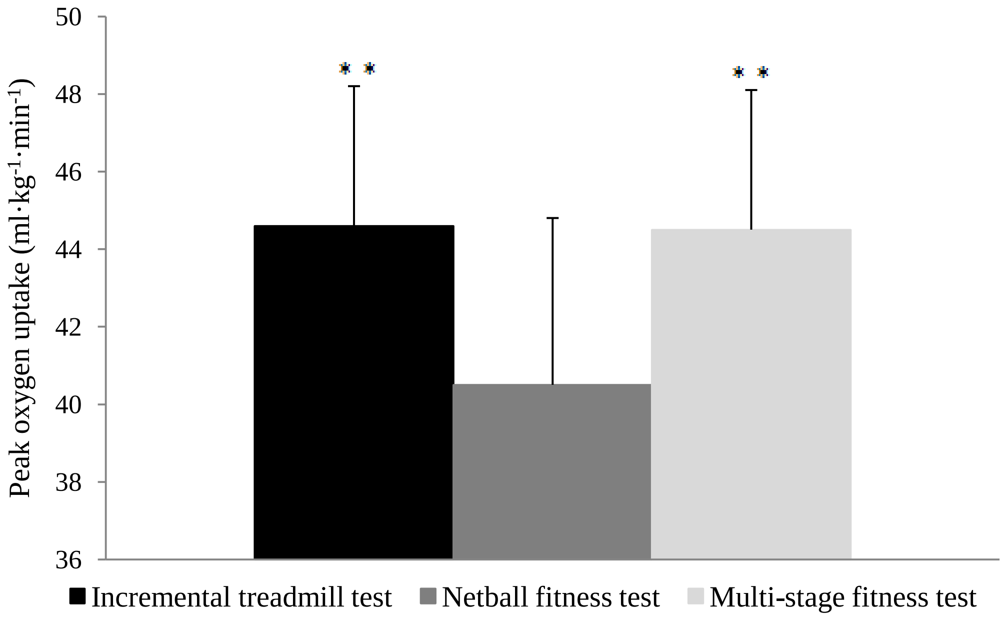


Figure 3.2 Mean ± SD $\dot{V}O_{2\,peak}$ values for all participants (n=12) for all three tests. **Significantly higher than those obtained from the netball fitness test (*p*<0.01).

3.3.2 Heart Rate and Respiratory Exchange Ratio at $\dot{V}O_{2\,peak}$

Mean ± SD values for heart rate at $\dot{V}O_{2\,peak}$ were not significantly different when the three tests were compared [$F(2,22)=3.394$, $p=0.052$] (table 3.3). Mean ± SD values for respiratory exchange ratio at $\dot{V}O_{2\,peak}$ derived from the incremental treadmill test and the netball fitness test were not significantly different [$F(1,11)=3.595$, $p=0.085$] (table 3.3).

Table 3.3. Mean ± SD heart rate and respiratory exchange ratio at $\dot{V}O_{2\,peak}$, for all participants (n=12), for each of the tests.

Parameters	Incremental treadmill test		Netball fitness test		Multi-stage fitness test	
	Mean	SD	Mean	SD	Mean	SD
Heart rate at $\dot{V}O_{2\,peak}$ ($b \cdot min^{-1}$)	198	10	195	7	201	13
Respiratory exchange ratio at $\dot{V}O_{2\,peak}$	1.18	0.08	1.12	0.06	N/A	N/A

3.3.3 Ratings of Perceived Exertion

Figure 3.3 shows that ratings of perceived exertion were higher in the multi-stage fitness test compared to the incremental treadmill test and the netball fitness test.

When the mean ± SD Pictorial Children's Effort Rating Table values at $\dot{V}O_{2\,peak}$ for each of the three tests were compared, there was a main effect of test [$F(2,22)=6.952$,

p=0.005]. Post-hoc Tukey tests identified that the Pictorial Children's Effort Rating Table values at $\dot{V}O_{2\,peak}$ for the multi-stage fitness test (10±1) were significantly higher compared to the incremental treadmill test (9±1, p<0.05, effect size=1.04) and the netball fitness test (8±1, p<0.01, effect size=2.09), respectively.

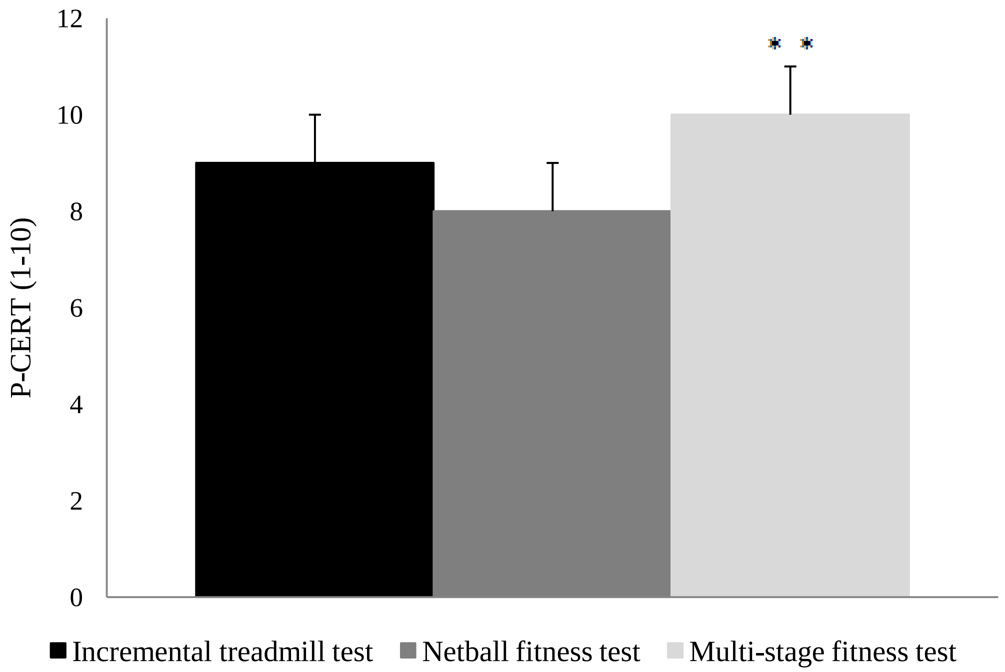


Figure 3.3. Mean ± SD Pictorial Children's Effort Rating Table (P-CERT) values at $\dot{V}O_{2\,peak}$ for all participants (n=12) for all three tests. **Significantly higher compared to incremental treadmill test (p<0.05) and netball fitness test (p<0.01).

3.3.4 Time to Reach $\dot{V}O_{2\,peak}$

There was a main effect of test on mean ± SD time to reach $\dot{V}O_{2\,peak}$ when the three tests were compared [F(2,22)=136.767, p<0.001]. Post-hoc Tukey tests revealed that all three tests differed significantly from one another. The girls took significantly more time to reach $\dot{V}O_{2\,peak}$ in the netball fitness test (20:57±3:19 min:sec) compared to the multi-stage fitness test (8:38±1:12 min:sec, p<0.01, effect size=5.33) and the incremental treadmill test (14:48±2:47 min:sec, p<0.01, effect size=2.23).

3.3.5 Test-Retest Reproducibility during the Netball Test

Test-retest coefficients of variation for the netball fitness test demonstrated good reproducibility for all parameters ($\dot{V}O_{2\,peak}$ 0.8 ml·kg^{-1}·min^{-1}, 3.5%; heart rate at $\dot{V}O_{2\,peak}$ 3 b·min^{-1}, 0.7%; respiratory exchange ratio at $\dot{V}O_{2\,peak}$ 0.01, 1.9%; time to reach $\dot{V}O_{2\,peak}$ 1:03 min:sec, 0.8%).

3.4 Discussion

The key finding of this study was that the time to exhaustion on the netball fitness test was significantly higher compared to the incremental treadmill test and the multi-stage fitness test. It was also identified that the $\dot{V}O_{2\ peak}$ values derived from the netball fitness test were significantly lower compared to values obtained from the incremental treadmill test and the multi-stage fitness test.

3.4.1 Application of the Field-Based Netball Fitness Test

These findings demonstrate the endurance performance capability of the netballers whilst completing the netball fitness test by the superior times to exhaustion (time to reach $\dot{V}O_{2\ peak}$) when compared to the incremental treadmill test and the multi-stage fitness test. This finding also suggests that something other than oxygen uptake is the limiting factor in the netball fitness test. Thus, indicating the ability of the netball fitness test to detect sport-specific capabilities of the adolescent netball players. The multi-stage fitness test and the incremental treadmill test are unable to detect these sport-specific differences since they purely measure linear aerobic capacity, not sport-specific aerobic capacity.

3.4.2 Specificity of the Netball Fitness Test

A further explanation for the sport-specific differences in $\dot{V}O_{2\ peak}$ derived from the three fitness tests is likely to be a result of the differing physiological demands of each of the tests (St Clair Gibson *et al.*, 1998). Treadmill running involves running at a constant, steady pace (St Clair Gibson *et al.*, 1998) and although the multi-stage fitness test involves an aspect of turning at the end of each shuttle run, an individual's performance on such tests is less affected by lack of skill and local muscular fatigue (Skinner *et al.*, 1971), which are more likely to influence performance during the netball fitness test. Continuous running is also not specific to the movement patterns, physiological demands, muscle actions and the skill element involved in netball. Netball is an intermittent sport with work periods interspersed with periods of rest (Allison, 1978). Young netballers require aerobic power to last the full duration of netball matches (40 minutes) (Loughran & O'Donoghue, 1999) and also need to restore ATP and oxygen stores during recovery periods following the intense periods of anaerobic activity (Allison, 1978; Otago, 1983), which occur when playing netball. The incremental treadmill test and multi-stage fitness test do not account for this anaerobic component or any additional physiological components and skill elements, such as strength, speed, power, agility, muscular endurance (McGrath & Ozanne-Smith, 1998) and choice reaction time (Gasston & Simpson, 2004). The netball fitness test incorporates various activities to account for these movement patterns, muscle actions and skill elements, such as backwards running (Loughran & O'Donoghue, 1999), side stepping (Steele & Chad, 1992), a foot agility task (Gasston & Simpson, 2004), jumping (Otago, 1983), changing direction and a choice reaction task (Gasston & Simpson, 2004).

Due to the anaerobic nature of the stages incorporated in the netball fitness test, type II muscle fibre recruitment and contribution to the exercise is likely to be higher, since such fibres have the ability to store larger quantities of phosphocreatine and therefore a higher capacity for glycolysis and glycogenolysis (Armstrong & Fawkner, 2007). Consequently, the dominant energy source for the quick bursts of activity during the netball fitness test such as the jumps is the high-energy phosphate system (Allison, 1978). According to Armstrong and Fawkner, (2007) increased type II muscle fibre recruitment results in an increased rate of pyruvate production due to the greater glycolytic enzyme content. Compared to type I muscle fibres, type II muscle fibres have fewer capillaries and mitochondria therefore a reduced capacity to process pyruvate aerobically, thus lactate is produced in the muscle. Due to the increased type II muscle fibre recruitment likely during the netball fitness test it is possible that there were fewer inactive adjacent muscle fibres available to process the excess lactate accumulation. Consequently, the body's ability to remove the lactate is impaired and there is an increased accumulation of lactate in the muscles and circulating blood. Ultimately this results in earlier exercise cessation due to local muscular fatigue (Skinner *et al.*, 1971). Therefore, although the adolescent female netballers players had higher times to exhaustion on the netball fitness test compared to the incremental treadmill test and the multi-stage fitness test in the present study, the anaerobic nature of the netball fitness test may still have been a possible reason for the lower $\dot{V}O_{2\ peak}$ values obtained using the netball fitness test, which led to the exercise cessation (at a lower ($\dot{V}O_{2\ peak}$).

Sampling of blood lactate concentrations post-exercise in all three fitness tests potentially could have provided further insight into the notion that local muscular fatigue was the limiting factor during the netball fitness test. Typically in adults, post-exercise blood lactate concentrations of above 8 mmol·L^{-1} are used to determine if an individual has reached their $\dot{V}O_{2\ max}$ during an exercise test to exhaustion (St Clair Gibson *et al.*, 1999). When young people are concerned it is inappropriate to set minimum post-exercise blood lactate values to identify when $\dot{V}O_{2\ peak}$ has been attained, as even when $\dot{V}O_{2\ peak}$ values are constant, blood lactate concentrations can vary (Fawkner & Armstrong, 2007). Since more appropriate subjective and physiological endpoints (see section 3.2.6) were used to determine when the adolescent girls had reached $\dot{V}O_{2\ peak}$ (Armstrong & Welsman, 2001) there was no justification initially to sample for post-exercise blood lactate concentrations. Despite lower $\dot{V}O_{2\ peak}$ values derived from the netball fitness test and a trend for the heart rate values at $\dot{V}O_{2\ peak}$ to be different between the three tests, the respiratory exchange ratio values at $\dot{V}O_{2\ peak}$ were not significantly different between the netball fitness test and the incremental treadmill test. Importantly, heart rate and respiratory exchange ratio were the two physiological indicators, amongst other subjective endpoints used to determine a maximum effort to exhaustion (Armstrong & Welsman, 2001) in all three of the fitness tests. Although there was a trend for heart rate values to be different between tests, all of the participants successfully attained all of the required criterions in all three tests, which according to Armstrong and Welsman (2001) indicates a maximal effort. A recent study by Barker et al. (2009) has suggested that the use of heart rate and the respiratory exchange ratio to identify a maximal effort in young people when completing $\dot{V}O_{2\ peak}$ tests may result in a sub-maximal $\dot{V}O_{2\ peak}$ being accepted. However, Armstrong and Welsman's (2001) criteria states that participants must demonstrate both subjective and objective indicators during $\dot{V}O_{2\ peak}$ tests in order for the effort to be deemed maximal, which was the case in all tests in the present study. However, the Pictorial Children's Effort Rating Table values support the notion that the differences in $\dot{V}O_{2\ peak}$ values between the netball fitness test, the incremental treadmill and the multi-stage fitness test were not due to a sub-maximal effort by the girls, as there were no significant differences in ratings of perceived exertion derived from the netball fitness test and the incremental treadmill test, despite a lower $\dot{V}O_{2\ peak}$ attainment in the netball fitness test.

Interestingly, the Pictorial Children's Effort Rating Table values at $\dot{V}O_{2\ peak}$ for the multi-stage fitness test were significantly higher compared to both the netball fitness test and the incremental treadmill test. This was the case despite the $\dot{V}O_{2\ peak}$ values from the multi-stage fitness test and the incremental treadmill tests being similar, 44.5±3.6 and 44.6±3.6 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. A possible explanation for this could be that since the levels for the multi-stage fitness test increased in 1 minute increments, no steady state in oxygen uptake was achieved. The 3 minute increments in levels during the incremental treadmill test and the netball fitness test enabled the adolescent netballers to reach a steady state in oxygen uptake (Fawkner & Armstrong, 2007) and therefore they may not have perceived these two tests to be as physically demanding compared to that of the multi-stage fitness test. With regards to the netball fitness test it is also likely that the girls perceived this test to be less exerting and easier to execute compared to the multi-stage fitness test as the girls were habituated to the muscle actions, movement patterns and physiological demands of the netball based activities included in the netball fitness test (Wilkinson, Leedale-Brown & Winter, 2008).

It is acknowledged that a limitation of the present study was the relatively small sample size (n=12), which has implications when interpreting significance values. For example, in the present study where there was a 'trend' in heart rate at $\dot{V}O_{2\,peak}$ (b·min^{-1}) to be different between the tests (p=0.052), but the p value was not actually <0.05, the level set for significance. It was not logistically possible to have used a larger sample size in the present study due to the strict criteria with regards to the type of participant required. A larger sample size may have influenced the significance of these findings. The present study required the recruitment of adolescent girls of the same gender (female) and maturation status, whilst also being involved with netball based activity 2-3 times per week.

Indeed, due to the amount of time the girls were asked to dedicate to this study (preliminary measures, familiarisation, completion of three fitness test), only four participants were used to explore test-retest reproducibility for $\dot{V}O_{2\,peak}$ (ml·kg^{-1}·min^{-1}), heart rate at $\dot{V}O_{2\,peak}$ (b·min^{-1}), respiratory exchange ratio at $\dot{V}O_{2\,peak}$ and time to reach $\dot{V}O_{2\,peak}$ (min:sec), for the netball fitness test. Although this may be interpreted as a possible limitation of the study, similar research in adults also used four participants to assess the test-retest reproducibility of an adult based netball specific fitness test (Gasston & Simpson, 2004).

3.5 Conclusion

In conclusion, the netball fitness test demonstrated its ability to detect sport-specific capabilities of the netball players since times to reach $\dot{V}O_{2\,peak}$ were superior compared to the multi-stage fitness test and the incremental treadmill test. The results suggest that the netball fitness test is a more meaningful test for assessing netball-specific aerobic power and endurance capability of adolescent female netball players.

Importantly, $\dot{V}O_{2\,peak}$ is often used to predict subsequent exercise intensity in studies requiring the investigation of exercise on other parameters, such as appetite and energy intake. Thus, the use of a sport-specific fitness test, such as the netball fitness test, as opposed to more general fitness tests (multi-stage fitness test and an incremental treadmill test), in exercise and appetite studies would ensure that a representative value for exercise intensity is obtained and therefore appetite and energy intake data is interpreted appropriately.

Similarly, in studies requiring the investigation of exercise on appetite and energy intake, it is also imperative that a robust method for assessing energy intake is established. This is especially important with regards to adolescent populations (due to inherent under-reporting issues) and also when exploring free-living energy intake following exercise.

CHAPTER 4

Agreement between Observers Recorded Energy Intake and Energy Intake from a Combined Self-Reported, Weighed Food Record and 24-Hour Recall Interview Technique, in Adolescent Netball Players

4.1 Introduction

In chapter three of this thesis, a method for assessing free-living, sport-specific $\dot{V}O_{2\ peak}$ in trained female adolescent netball players was evaluated in order to allow the prediction of subsequent exercise intensity, in studies exploring the effect of exercise on parameters such as exercise and appetite. The information regarding the exercise intensity of exercise bouts imposed in such exercise and appetite studies, enables appetite and energy intake data to be interpreted appropriately. Therefore, it is also imperative to establish robust methods for assessing free-living energy intake for use in exercise and appetite research studies, in paediatric populations, in order for energy intake data following exercise (depending on exercise intensity) to again be interpreted appropriately.

The most frequently used methods to assess energy intake under free-living conditions in young people include observation, self-reported estimated or weighed food records, retrospective 24-hour recall interviews and food frequency questionnaires (Dodd, 2007). Initial studies which explored energy intake and appetite responses to exercise in young people over very short-term time periods of one hour (Rumbold & Dodd, 2007) and one day (Moore *et al*., 2004) used observation. However, when energy intake data is required to be collected over a period longer than one day and in a free-living environment, it is not realistic to use observational methods. Consequently, self-reported estimated or weighed food records and retrospective 24-hour recall interviews are the preferred methods of choice when assessing energy intake over several days and indeed have been used successfully to assess energy intake following cycling exercise in young girls (10-11 years) over 5-days (Dodd, Welsman & Armstrong, 2008). Although the study by Dodd, Welsman and Armstrong (2008) was laboratory-based the majority of the time, there was a free-living element when the young girls were required to record their food intake in a diary and were then interviewed the following morning regarding their food intake in the previous 24-hour period. Unfortunately, Dodd, Welsman and Armstrong (2008) did not assess the accuracy of their chosen energy intake collection technique, which with regards to free-living exercise and appetite studies is imperative.

As described in the literature review (see section 2.3.1), food records are regarded as the 'gold standard' technique used to assess dietary intake (Black *et al*., 1991; Ashley & Bovee, 2003). However, when self-report weighed food diaries have been validated against doubly labelled water (see section 2.3.1), an inherent problem is under-reporting of energy intake in young people (Dodd, 2007). This is particularly the case in normal-weight adolescent girls of increasing age (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003) and increasing body mass index (Livingstone *et al*., 1992b; Bratteby *et al*., 1998). In this special population energy intake is likely to be underreported by approximately 20% (Bandini *et al*., 1990; Bratteby *et al*., 1998) to 30% (Livingstone *et al*., 1992b; Bandini *et al*., 2003) when self-reported weighed food diaries are utilised.

Interestingly, however, when the retrospective technique of 24-hour recalls has been used to assess energy intake in adolescent girls, they have been found to provide a valid estimate of energy intake on a group level (Greger & Etnyre, 1978). The majority of studies which have examined the validity of 24-hour recall interviews as a means of assessing energy intake have however only explored a portion of the day or a single test meal and not a 24-hour period (McPherson *et al.*, 2000). Only six studies have investigated a 24-hour period (Greger & Etnyre, 1978; Van Horn *et al.*, 1990; Mullenbach *et al.*, 1992; Lytle *et al.*, 1993; Johnson, Driscoll & Goran, 1996; Lindquist, Cummings & Goran, 2000). When the reference standard of doubly labelled water has not been available, several of these studies have used actual measured energy intake as a reference technique to compare reported energy intake to, successfully in children (Lytle *et al*., 1993) and adolescent girls (Greger & Etnyre, 1978).

It is well recognised that cognitive ability plays a major role in an individual's ability to perform well on a 24-hour recall interview (Baranowski & Domel, 1994; Foster *et al.*, 2008b). Since adolescents have fully developed cognitive capabilities and possibly more understanding regarding food preparation than their younger counterparts (Livingstone & Robson, 2000), their ability to perform well on a 24-hour recall interview is likely to be optimal. Indeed, Foster et al. (2008b) identified that the precision and accuracy of portion size estimation during a 24-hour recall increases with age, with younger children (4-10 years old) tending to overestimate portion sizes and are therefore less accurate and less precise compared to older children and adolescents (11-14 years old).

An issue which influences the accuracy of energy intake data, in normal weight adolescent girls, is dissatisfaction with body weight (Wardles & Beales, 1986), body image and body shape (Livingstone & Robson, 2000). Such preoccupations with body weight, image and shape typically lead to adolescent girls restricting their energy intake either consciously or subconsciously (Livingstone & Robson, 2000) to lose or maintain body weight, which is known as dietary restraint (Herman & Polivy, 1980). However, such dietary restraint issues need to be investigated in trained adolescent girls, as the possibility of under-reporting due to pre-occupations with body weight or image (Livingstone *et al*., 1992b) may be reduced in this population. Livingstone and Robson (2000) suggest that it is important to identify adolescent girls who are likely to mis-report as a result of dietary restraint. The Dutch Eating Behaviour Questionnaire (Van Strein *et al*., 1986a) provides a measure of dietary restraint which is appropriate for use with children as young as 9 years (Hill & Robinson, 1991) and also adolescent girls (11.8-18.0 years old) (Wardle *et al*., 1992).

Livingstone and Robson (2000) suggest that estimates of energy intake can be enhanced when two methods of dietary assessment are used conjunctively. As described in the literature review, previous studies have shown that the use of 24-hour recall interviews combined with a non-quantified food record, which was used as a memory prompt during a 24-hour recall interview, overestimated energy intake in young children (8 years old) (Lytle *et al.*, 1993). Accurate and precise portion size estimation was imperative in this study, however it has been identified that young children are less accurate and less precise than older children and adolescents (11-14 years old) at estimating portion size, with a tendency for overestimating portion sizes during a 24-hour recall interview (Foster *et al*., 2008b). Thus this provides a possible explanation for the overestimation in energy intake which was identified by Lytle et al. (1993). Therefore, when choosing energy intake assessment techniques to be used conjunctively, consideration of the age of participants is imperative.

Thus in the case of assessing free-living energy intake in normal-weight trained adolescent girls it seems appropriate that self-reported weighed food diaries are used as the main energy intake assessment technique, alongside 24-hour recall interviews. It is acknowledged that normal-weight adolescent girls of increasing age tend to underreport their energy intake using self-reported weighed food records (Livingstone *et al.*, 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003), however no study has investigated the use of a combined self-reported, weighed food record and 24-hour recall interview technique in a group of trained adolescent girls. Consequently, the aim of this study was to explore the agreement over 24-hours between observer recorded energy intake (MJ) and energy intake (MJ) from a combined self reported weighed food record and 24-hour recall interview technique in adolescent netball players.

4.2 Method

4.2.1 Design

This study determined the accuracy with which adolescent girls weighed, reported and recalled their food and drink intake, over a 24-hour period (12-hours in the nutrition kitchen of Northumbria University and 12-hours in a free-living environment).

4.2.2 Participants

The study was approved by the School of Psychology and Sport Sciences Research Ethics Committee at the University of Northumbria. Written informed consent was obtained from both the girls and parents or guardians prior to data collection. Both parties were provided with information sheets detailing the procedures and requirements of the study. The girls were informed that the study was based on general food intake analysis, but not told that it was concerned with their ability to record their food and fluid intake.

All girls aged 10-16 years old who attended a netball club in the North East of England were invited to participate in the study. Following an informal talk which the research team provided to all of the club members, 13 girls aged 14-16 years old volunteered to participate on the proposed testing date. The participants were required to participate in netball-based exercise or competition at least three times per week, in order to be classified as trained with regards to netball. The Dutch Eating Behaviour Questionnaire (Van Strein *et al.*, 1986a) was administered to determine whether the participants were restrained or unrestrained eaters. All 13 girls were classified as unrestrained eaters, with the mean (±SD) 2.13±0.62 dietary restraint score falling into the average category for high school females.

Eleven out of the 13 girls who volunteered to participate in this study were also participants in the study described in chapter 5.

4.2.3 Body Composition Assessment

Body composition was assessed as per section 3.2.3.

4.2.4 Maturity Offset

Maturity offset was assessed as per section 3.2.4.

4.2.5 Study Protocol

The study was carried out over one weekend. The participants spent 12-hours at Northumbria University, from 08:00 in the morning to 20:00 in the evening. During the day the participants were kept occupied by participating in various activities, such as playing board games, watching DVD’s and completing various art and craft activities. They were provided with breakfast, lunch and dinner, along with snacks throughout the day, which they were able to help themselves to. For the period between 20:00 and 08:00 the following morning, the participants were allowed to take any food items from the university laboratory home. Researchers liaised with parents and or guardians of the participants to ensure that they did not consume foods from alternative sources and that any leftover food was returned to the research team the next morning during the 24-hour recall interview.

4.2.6 Food and Drink Items Served

Importantly, the food and drink items selected for inclusion in the study were based on a food preference questionnaire which was administered prior to the study. This questionnaire was based on dietary information derived from self-reported, weighed food diaries and 24-hour recall interviews (see chapter five). This ensured that all food and drink items provided were palatable and typical of what the participants consumed at home and school on a regular basis. A wide variety of foods were offered to the children at each meal in order to replicate what would typically occur in a free living situation. Details of foods offered are provided in table 4.1.

Table 4.1. Foods offered to the participants during the study day.

Meal	Food and drink items
Breakfast	Coco pops, rice krispies, rice krispies multigrain, coco pop munchers, frosties, cornflakes, semi-skimmed or whole milk.
Lunch	Tuna and sweetcorn mayonnaise sandwiches, white/brown bread, with/without margarine. Ham sandwiches, white/brown bread, with/without margarine.
Dinner	Tomato pasta (with/without cheese). Jacket potato (with/without beans, cheese, margarine, tomato sauce). Chicken and tomato cous cous.
Secondary Items	Orange squash (no added sugar), diet coke, pure apple and orange juice, fruit (apples, clementines, bananas), yoghurt, cereal bars, crisps, confectionary.

4.2.7 Participant Reported Energy Intake (Self-Reported, Weighed Food Diaries)

All the food and drink items were numerically coded. The participants could collect food and drink items at their discretion throughout the day, although they were instructed to inform a member of the research team of the numerical code of each item collected. Breakfast and lunch, food and drink items, which needed to be kept chilled, were stored in a refridgerator, whilst other breakfast and lunch items were stored in cupboards. However, with regards to dinner items the girls picked what they wanted off the menu and were provided with freshly cooked, known quantities of each dinner item they chose.

It was explained to the girls that every item of food and drink should be weighed or measured prior to consumption and likewise, if there was any leftover food or drink, this should also should be weighed or measured. It was emphasised that food and drink items should be reported in their individual food diaries provided. To facilitate this recording process, each participant was issued with a set of dietary scales (Gormet White Electronic Scales, Hanson), a measuring jug and a designated table where any leftover food or drink items were collected. The participants were taught how to use the scales and measuring jugs and were also given a detailed explanation of what information should be reported in the food diaries. These instructions were also provided in the food diaries for the participants to refer to (see appendix C for an example of the food diary). Therefore, throughout the day after the participants collected a food or drink item they weighed the quantity and wrote the food or drink item in their diary along with the time of consumption and the amount consumed.

4.2.8 Observer Reported Energy Intake

In parallel with the participants recording their food and drink intake in their diaries, all food and drinks were prepared and covertly pre-weighed or measured by the research team to the nearest gram or millilitre. Each food and drink item was given a numerical code in order for the researchers to identify the exact nutritional content. When participants collected food and drink items to consume, they were instructed to inform a member of the research team of the numerical code. This was then recorded to correspond with the correct participant. The research team collected leftover food and drink items, in order to covertly weigh and dispose of them appropriately. Subsequently, this facilitated the calculation of total daily energy intake for each participant.

4.2.9 24-hour Recall Interviews

The following morning, after the study day, the two researchers who looked after the participants whilst they were at the university, visited each of the participants at home to complete a retrospective 24-hour recall interview. This involved conducting individual, face-to-face interviews with each girl using a two-pass method (Ashley & Bovee, 2003). This took approximately 15-20 minutes per participant. First, the overall eating events of the previous day were reviewed to identify the main foods and beverages consumed. Secondly, the participants were prompted for more information, such as condiments, brand names, additional food or drink items, how the foods were prepared and cooked, and portion sizes if not provided.

4.2.10 Estimation of Energy Intake

To calculate the observer and participants total daily energy intake ($MJ \cdot d^{-1}$), the nutritional content of all food and drink items was obtained using the food packaging. Although portion sizes were based on portion sizes described in chapter five. When food portions were not identified by the participants, amounts were first substituted using a portion size recorded in the individual's food diary corresponding to the same food or drink item. If such a substitute was not available, standard portion estimates from the Dietmaster Software Package were used [Dietmaster (1999) Version 4.0 Professional Edition, Swift Computer Systems].

4.2.11 Statistical Analysis

Data are presented as mean ± SD. The agreement between estimates of energy intake (MJ) reported by the partcipants (self-reported, weighed food diaries and 24-hour recall interviews) and by the observers was assessed using the Bland and Altman (1986) method. This required a pair-wise comparison, showing the relative bias (mean difference), random error (1.96 SD of the difference) and consequently the limits of agreement (mean difference ± 1.96 SD of the difference) between participant reported energy intake and researcher reported energy intake, by plotting the difference between the methods against the mean of the two estimates, as recommended by Livingstone et al. (1992b).

4.3 Results

The age and physical characteristics (mean ± SD) of the participants were: age 15.5±0.6 years; height 1.68±0.06 m; weight 61.1±8.0 kg; BMI 21.6±2.5 kg/m^2; and body fat 21.5±6.9%. The maturity offset was 2.7±0.5 years from peak height velocity, indicating that all the girls had reached their predicted peak height velocity (positive maturity offset) and thus were of a similar maturation status.

The level of agreement between energy intake reported by participants and energy intake reported by observers was investigated by plotting the individual differences between the two energy intake measurement techniques against the mean of both values. Mean energy intake reported by the participants and mean energy intake reported by the researchers were 9.56±2.96 and 9.11±2.60 $MJ \cdot d^{-1}$, respectively. Therefore, the mean difference between the two measurement techniques was 0.46 $MJ \cdot d^{-1}$ or the change in mean expressed as a percentage was 4.2%, indicating that the combined self-reported, weighed food diary and 24-hour recall interview technique has a slight bias towards over-reporting energy intake. Figure 4.1 shows that the random error equated to 1.5 MJ (1.96 SD of the mean difference), therefore the 95% limits of agreement (mean difference between participant and researcher reported energy intake ± random error) was large, ranging from -1.04 to +1.96 $MJ \cdot d^{-1}$. This indicated poor agreement between the two measurement techniques on an individual level, with individual differences between participant and researcher reported energy intake ranging from -8.6% to 20.3%. The 95% confidence interval for bias however ranged from 0.00 to 0.92 $MJ \cdot d^{-1}$, which indicated good agreement at the group level.

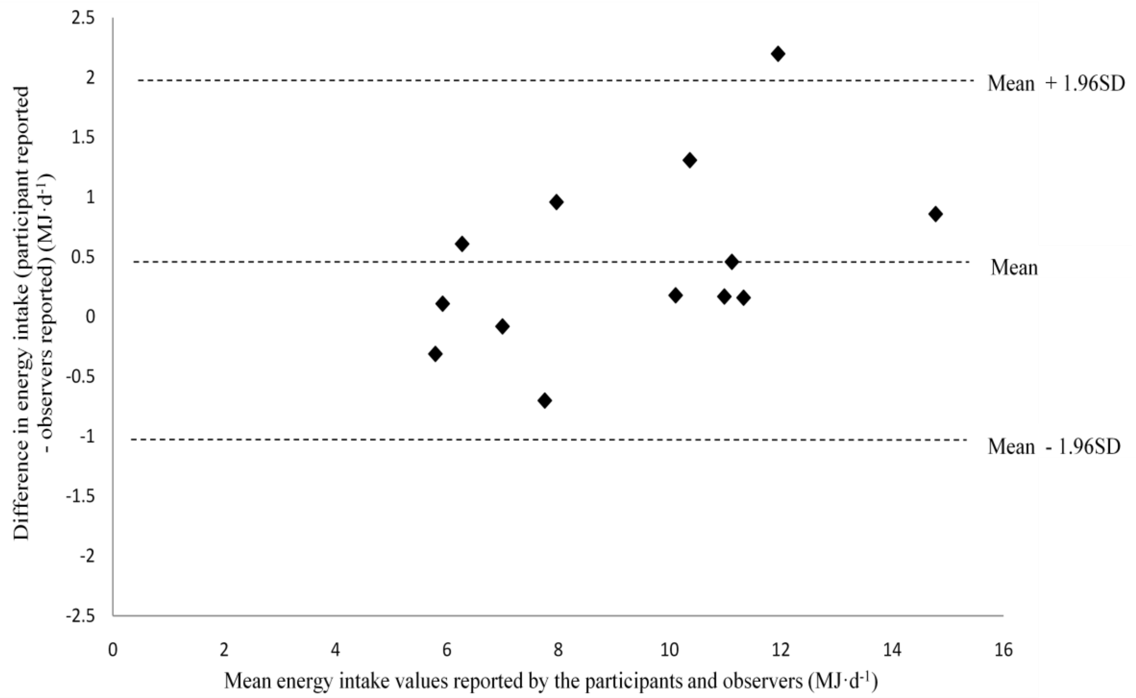


Figure 4.1. Individual differences in energy intake (energy intake reported by participants – energy intake reported by observers) plotted against the mean of the measurements for energy intake.

4.4 Discussion

This study was the first to investigate the agreement between observer reported energy intake and energy intake from a combined self-reported, weighed food diary and 24-hour recall interview technique in a group of trained adolescent netball players aged 14-16 years old. The results of this study suggest that at a group level, the combined use of self-reported weighed food diaries and 24-hour recall interviews is an effective method for quantifying energy intake in participants of this age.

When the mean energy intake value reported by the researcher was compared to the mean energy intake value reported by the participants there was a slight bias towards an over-reporting of energy intake in the group of trained adolescent girls by an average of 4.2%. This finding was unexpected since previous studies using doubly labelled water as the reference technique identified a tendency for normal-weight adolescent participants to underreport their food intake with increasing age (Livingstone *et al.*, 1992b; Bratteby *et al.*, 1998; Bandini *et al.*, 2003). Importantly, Livingstone et al. (1992b), Bratteby et al. (1998) and Bandini et al. (2003) all employed a singular 7 day food diary technique. Initially, Livingstone et al. (1992b) found that boys and girls aged 7 and 9 years old could quantify their energy intake accurately when compared to simultaneous measures of energy expenditure using doubly labelled water. However, this was not the case for the 12, 15 and 18 year old children and adolescents, with accuracy declining with increasing age. A similar pattern was identified in Swedish adolescent girls and boys aged 15 years (Bratteby *et al.*, 1998) and in a longitudinal study by Bandini et al. (2003) who also identified a decline in accuracy with increasing age when the girls were studied at age 10, 12 and 15 years old. However, when compared to the literature using similar aged participants, the findings of the present study demonstrate that at least on a group level the combined use of two dietary measurement techniques (self-reported, weighed food diaries and 24-hour recall interviews) facilitates 'acceptable' quantification of energy intake in adolescent girls. Thus in an attempt to make a judgement about an 'acceptable' level of bias with regards to self-reported energy intake, Livingstone et al. (1992b) reported 'good' agreement at a group level when 95% confidence interval values for energy intake (measured using diet histories) compared to doubly labelled water, fell between +0.05 and +0.85 $MJ \cdot d^{-1}$, which is in line with the values identified in the present study (0.00 to +0.92 $MJ \cdot d^{-1}$).

Mean total daily energy intake values derived from this study were above the estimated average requirements proposed by the Department of Health (1991). These guidelines suggest girls aged 14-16 years old should be consuming between 7.72 and 8.83 $MJ \cdot d^{-1}$, whereas the results from the present study demonstrate that the girls consumed in a single day 9.11±2.60 $MJ \cdot d^{-1}$, which is slightly above these figures, although in agreement with mean daily energy intake values found in chapters five and six. However, the recommended values are estimated requirements of energy intake for groups of the general population, with approximately 50% of the population requiring less and 50% requiring more and therefore do not account for elevated physical activity levels. Indeed, the participants involved with this study were trained adolescents with regards to netball and thus require more energy from food compared to their sedentary counterparts (Montfort-Steiger & Williams, 2007), primarily in the form of carbohydrate (A.D.A, 1996). Since the girls were offered food *ad libitum* there is the possibility that they ate excessive amounts of carbohydrate. Indeed, the main contributor in terms of macronutrient contribution to daily energy intake in the present study was carbohydrate (73±4%), followed by protein (16±3%) then fat (11±3%). The values derived for daily energy intake and macronutrient content of the diet are comparable to values identified in chapters five and six, when free-living energy intake was assessed in trained adolescent girls. This indicates that the participants did not alter their food intake habits during the present study day and also demonstrates that the food and drink items and portion sizes provided were representative of habitual energy intake in free-living conditions.

4.4.1 Group Selection

Explanations for an over-reporting of food intake may relate to the participant group selection. All of the participants were trained individuals (as intended) and according to the Dutch Eating Behaviour Questionnaire scores all participants were classified as unrestrained eaters. This reduces the possibility of underreporting of energy intake due to dietary restraint as a symptom of pre-occupations with body weight or image, as has been suggested in previous studies of this nature (Livingstone *et al.*, 1992b). Further support for this comes from the healthy BMI values (provided in the results section of this chapter), according to age and gender classified by Cole et al. (2000). It is more likely that such participants are committed when it comes to reporting energy intake. Being inherently generally motivated, as athletes often are (Gould, 1982), is also an important quality for individuals to possess when weighing and recording food intake (Foster *et al.*, 2008a). It must also be noted that 11 out of the 13 participants who took part in the present study were also involved in the following study of this thesis (chapter 5). Indeed, Livingstone et al. (1992b) suggests that a detailed explanation and demonstration of the food weighing and recording process should be conducted prior to exercise and appetite research studies, providing the opportunity for participants to practice this technique in the presence of the researcher. However, it was also the intention of this work to explore the energy intake reporting accuracy of the participants (chapter 4) to inform later intervention studies (chapter 5).

4.4.2 Phantom Foods

A likely explanation for the over-reporting of energy intake by the adolescent participants in this study day may also relate to the notion of 'phantom foods' first introduced by Meredith et al. (1951). Phantom foods referred to food and drink items which the participants recorded and recalled and therefore believed they had consumed when actually they may not have consumed. The mean ± SD MJ content of the phantom foods equated to 0.25±0.30 $MJ \cdot d^{-1}$, which given that the participants over-reported their energy intake by 0.46 $MJ \cdot d^{-1}$, phantom foods therefore explained 54.3% of the over-reporting of energy intake. The majority of the phantom foods were secondary items (92.9%), therefore these findings correspond with those of Emmons and Hayes (1973) who suggest that these items are more difficult to record and recall and that secondary food and drink items eaten on days close to the recording period are often likely to be recorded and recalled instead.

The notion of phantom foods may also explain the large between individual variability identified using the limits of agreement analysis (figure 4.1), with five out of 13 of the participants over-reporting their energy intake. Indeed, the large between individual variability identified in figure 4.1, clearly demonstrates how difficult it is for researchers to assess a participants energy intake as the amount of bias changes dependent on whether energy intake is measured on a group or individual basis (figure 4.1). However, it would be extremely logistically demanding to assess energy intake on an individual level in free-living exercise and appetite research studies. Thus similar to other studies of this nature in adults, a group value for bias in relation to the specific population, in this case trained, female adolescent netball players, is typically reported.

4.4.3 Over-Reporting of Food and Drink Items

The remaining 0.21 $MJ \cdot d^{-1}$ (45.7%) of the 0.46 $MJ \cdot d^{-1}$over-reporting of energy intake by the participants resulted from over-reporting of secondary food and drink items (41%), dinner items (27.9%), breakfast items (21.3%) and lunch items (9.8%) respectively. Through observation on the study day two issues arose regarding the use of the dietary scales. The comparison between weighed food intake by the participants and researcher weighed food intake indicated that the participants were accurately weighing and reporting food and drink items prior to consumption but on occasions they failed to weigh and report left over amounts in their food diaries. Close examination of the data indicated that participant number eight over-reported their energy intake the most (20.3%) by failing to weigh a left over primary tea item. Additionally, participants may not always have set the scale back to zero when weighing food items. Secondary food items were consumed throughout the day and quite often alongside other secondary food and drink items, which may have increased the likelihood of the scales not being reset to zero, due to haste of the participants.

4.5 Conclusion

This is the first study to explore the agreement between observer reported energy intake and energy intake from a combined self-reported, weighed food diary and 24-hour recall interview in trained adolescent girls. Free-living exercise and appetite studies use the food diary method often, without a second method such as the 24-hour recall interview. Previous exercise and appetite studies in young people have been acute and laboratory based, therefore observation of food intake was possible, however there is now a need for more longer-term studies. The combined self-reported, weighed food diary and 24-hour recall interview technique provides a means for assessing energy intake over more longer-term periods. In the present study, the combined self-reported, weighed food diary and 24-hour recall interview, was shown to be an effective method to employ on a group basis, when quantifying energy intake in a group of trained adolescent girls (14-16 years old), demonstrated by the 'acceptable' 95% confidence interval values (0.00 to +0.92 $MJ \cdot d^{-1}$), which are in line with other paediatric studies of this nature (Livingstone *et al.*, 1992b).

In chapters three and four of this thesis so far, robust and appropriate methods for assessing sport-specific exercise intensity, energy expenditure and also energy intake using a combined self-reported, weighed food diary and 24-hour recall interview technique, in groups of adolescent populations were established. These methods can therefore be used in the subsequent two studies (chapters five and six) of this thesis in in order for appetite and energy intake data following exercise (depending on exercise intensity) to be interpreted appropriately.

CHAPTER 5

Energy Intake and Appetite Following Netball Exercise over 5-days, in Trained 13-15 year old Netball Players

5.1 Introduction

In chapter four of this thesis, a combined method of self-reported, weighed food diaries and 24-hour recall interviews was established, to assess the free-living energy intake of trained female adolescent netball players in exercise and appetite studies, as accurately as possible. In addition to this, in chapter three of this thesis a method for assessing free-living, sport-specific activity levels in trained female adolescent netball players was established in order to predict the intensity of exercise protocols in energy regulation and appetite studies as accurately as possible. Taken together these methods provide a strong basis for accurately assessing free-living exercise intensity and energy intake in order to explore the effect of exercise on energy intake and appetite in young people.

Young people need to maintain a slight positive energy balance of approximately 1-2% in order to sustain healthy growth and development (Livingstone, Robson & Totton, 2000). The energy cost of such growth and development is estimated to be 5 Kcal/g of tissue gain (Millward, Garlick & Reeds, 1976). The assessment of young people's energy expenditure in a free-living environment is important in order to determine such energy balance status.

The other major contributing factor to energy balance is energy intake. When considering energy intake measurement techniques, methods should be robust to minimise error resulting from under-reporting of energy intake, which is typically the case in normal-weight adolescent girls (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003). In chapter four of this thesis, the combined self-reported, weighed food diary and 24-hour recall interview technique was an effective method to employ on a group basis, when quantifying energy intake in a group of trained adolescent girls (14-16 years old). Increasingly in exercise and appetite studies in adults, researchers have also assessed mood parameters (Stubbs *et al*., 2002b; Stubbs *et al.*, 2004). No exercise and appetite studies involving paediatric populations have investigated mood. Mood has been demonstrated to be closely associated with food cravings in adult women (Hill, Weaver & Blundell, 1991) and therefore to account for any fluctuations in food intake in the present study of adolescent girls, investigation of mood is warranted. Available VAS to assess mood are those developed by Bond and Lader (1974), although it must be recognised that these scales were initially used to explore the effects of different drugs in adults. Thus, for the purpose of the present study the terminology of the VAS were adapted to suit the age of the participants and the nature of the research.

The short-term relationship between exercise (and thus exercise-induced energy expenditure) and subsequent energy intake is presently not established in paediatric populations. To maintain energy balance, lean young people must compensate through energy intake for exercise-induced energy expenditure at some point, since such a marked negative energy balance would result in continual weight loss (Dodd, 2007). The majority of work to date in paediatric populations has explored only the impact of dietary manipulations on subsequent energy intake (Wilson, 1999). Results are equivocal, with some authors identifying a lack of compensatory down-regulation of energy intake when energy intake is manipulated (Wilson, 1991; Wilson, 1994; Hägg *et al*., 1998; Wilson, 1999) others identifying a compensatory down-regulation of energy intake (Birch & Deysher, 1985; Birch & Deysher, 1986; Birch, McPhee & Sullivan, 1989; Hetherington, Wood & Lyburn, 2000), and other studies identifying a partial down-regulation of energy intake (Birch *et al*., 1993; Hägg *et al*., 1998; Zandstra *et al*., 2000; Warren, Henry & Simonite, 2003; Johnson & Taylor-Holloway, 2006; Cecil *et al*., 2006). It is of interest therefore to investigate whether a similar response following energy intake pre-loads is identified when energy expenditure is manipulated instead, by imposing a bout of exercise.

There is a dearth of information concerning young people and their responses to exercise in terms of energy intake. The first published study involving 19 lean girls (9-10 years old), conducted by Moore et al. (2004), intervened with two laboratory-based cycling protocols of 50% $\dot{V}O_{2\,peak}$ and 75% $\dot{V}O_{2\,peak}$, eliciting 1.5 MJ energy expenditure, over a 1-day period. Moore et al. (2004) found no increase in energy intake in response to the prescribed exercise. A follow-up study which employed similar high intensity laboratory-based cycling exercise over 2-days, but with a longer energy intake monitoring period totalling 5-days was subsequently conducted by the same group (Dodd, Welsman & Armstrong, 2008). No differences were identified for total daily energy intake or total 5-day energy intake for either lean or overweight girls (11.5±0.4 years old).

As described in the literature review (see section 2.5), laboratory-based protocols are a popular means of manipulating energy expenditure, however they lack specificity as they fail to represent the habitual exercise patterns of young people. It has been suggested that intermittent activities are more attractive and motivating to young people (Ratel *et al.*, 2004) increasing levels of conformity and participation (Livingstone, Robson & Totton, 2000). In schools a major element of the National Curriculum for Physical Education encourages teachers to deliver sporadic game-based activities, such as netball and football. The dearth of studies which have employed appropriate free-living exercise interventions have manifested themselves in sport specific short-term intervention studies. At present these only include athletic activities (Verger, Lanteaume & Louis- Sylvestre, 1992; 1994) and swimming (Verger *et al*., 1992) using adult groups. The methodologies employed in these studies have lacked precision in terms of study design and energy expenditure measurement techniques. There is also some inconsistency with findings. Initially, Verger et al. (1992) identified that there is an increase in carbohydrate consumption following athletic activities when compared to a sedentary condition. However in a similar study design there was an increase in the percentage of daily energy intake as protein as opposed to fat and carbohydrate (Verger, Lanteaume & Louis- Sylvestre, 1994). Free-living macronutrient consumption following exercise has yet to be explored in paediatric groups.

Following the prescription of sport-specific exercise, which was representative of the physical activity patterns of young girls (Rumbold & Dodd, 2007), and the lack of energy intake compensation identified in paediatric studies so far (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008) such baseline work should now be extended into the free-living environment, over an extended duration of 5-days, to correspond with the school week. Habitual energy intake, time to onset of eating and appetite responses to representative exercise as opposed to laboratory based exercise protocols, which have been used in previous studies (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008) should now also be explored, which allow adolescent girls to control what and when they eat (Moore *et al.*, 2004). To date trained lean adolescent girls have not been considered in energy regulation and appetite studies and it would be assumed that energy intake compensation following exercise would occur in a population group with relatively high energy expenditure levels. In addition, previous paediatric studies have only monitored energy intake, macronutrient intake, energy expenditure, energy balance and appetite (hunger, prospective food consumption and fullness) over one maintenance day prior to the exercise intervention (Moore *et al.,* 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008).

Consequently, in a group of lean, trained adolescent girls (13-15 years old) the main aims of the present short-term intervention study were to:

- Impose two maintenance days prior to a NSEP and SED intervention, and monitor free-living, 24-hour energy intake, macronutrient intake, 24-hour energy expenditure, 24-hour energy balance, subjective appetite (hunger, prospective food consumption and fullness) and mood (alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood).

- Investigate subjective appetite (hunger, prospective food consumption and fullness) and mood (alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood) before versus after a NSEP intervention and also compared to a SED intervention.
- Explore the time to onset of eating following a NSEP and SED intervention.
- Over 3-days following both a NSEP and SED intervention, explore free-living, 24-hour energy intake, 24-hour energy expenditure, 24-hour energy balance, daily macronutrient selection (fat, carbohydrate and protein), daily subjective appetite (hunger, prospective food consumption and fullness), daily mood (alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood), total 2-day (48-hour) energy intake and 2-day (48-hour) energy balance, total 3-day (72-hour) energy intake, 3-day (72-hour) energy expenditure and 3-day (72-hour) energy balance.

Overall the aim of this study was to establish whether free-living energy intake and subjective appetite would be influenced by a bout of netball specific exercise in female adolescent netball players, over 3-days.

5.2 Method

5.2.1 Design

Using a within-subjects, randomised cross over design, the study compared energy intake over 5-days (two maintenance days and three follow-up days) following a NSEP and a SED intervention.

5.2.2 Participants

The study was approved by the School of Psychology and Sport Sciences Research Ethics Committee at the University of Northumbria. Written informed consent was obtained from both the children and parents or guardians prior to data collection. Both parties were provided with information sheets detailing the procedures and requirements of the study. They were informed that the study was based on general diet and exercise but not told that it was concerned with specific energy intake, following an exercise bout.

Eleven girls aged 13-15 years old (physical characteristics provided in results section), were recruited from a local community based netball club and were divided into groups of three or four and remained in these groups for all testing procedures. Inclusion was based on four criteria. Firstly, the participants were required to habitually participate in netball-based exercise or competition at least three times per week, in order to be classified as trained with regards to netball. Researchers liaised with the netball coaches and school physical education teachers at the respective school to ensure this. Secondly a medical questionnaire was administered prior to the study. This was to identify any injuries, which potentially would have prevented completion of the NSEP and also any illnesses, which may have affected food intake. In the third instance, only those participants with a healthy BMI (kg/m^2) score were able to participate in the study. This was determined using the age and gender-specific classification of Cole et al. (2000) for the United Kingdom, where BMI scores which fell between the 9th centile and the 91st centile were accepted as an indicator of a healthy BMI. Fourthly, all the participants were classified as unrestrained eaters, using the Dutch Eating Behaviour Questionnaire (Van Strein *et al.*, 1986a), with the mean (±SD) 2.13±0.63 dietary restraint value falling into the average category for high school females. Any participants who were unable to attend any of the testing dates were also excluded.

5.2.3 Study Protocol

The first 5-day treatment week (A) (09:00 hours Sunday – 09:00 hours Friday) commenced 7 days after two preliminary visits had been completed. The second treatment week (B) followed the first, 2 weeks later as shown in table 5.1. For logistical reasons, the participants were randomly allocated to three groups, one group of three and two groups of four. Thus the full protocol described here was conducted three times to accommodate all participants.

Preliminary week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Day 1 – Familiarisation, body composition assessment, FLEX heart rate and peak oxygen uptake assessment. Day 2 – Resting metabolic rate measurement.	Treatment week A: NSEP or SED intervention.	Wash out week.	Wash out week.	Treatment week B: NSEP or SED intervention.

Table 5.1. Study protocol.

5.2.4 Preliminary Measures: Day 1

Familiarisation

The aims of this session were three-fold. Firstly, the participants became familiar with the completion of the VAS to assess appetite (hunger, prospective food consumption and fullness) and mood (alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood) and were provided with a VAS diary. Secondly, a detailed explanation and demonstration of the food weighing and recording process was conducted, providing the opportunity for the girls to practice this technique in the presence of the researcher (Livingstone *et al.*, 1992b). Using individual food diaries, the participants were encouraged to record the name of the food item, the time of ingestion, the method of preparation and the amount of food before and after consumption. Lastly, the participants were habituated to the NSEP and also heart rate monitors and receivers (Polar RS-400 and S610i, Heart Rate Monitors, Polar, O.Y. Finland).

Body Composition Assessment

Body composition was assessed as per section 3.2.3.

FLEX Heart Rate and Peak Oxygen Uptake Assessment

In order to estimate energy expenditure a FLEX heart rate calibration was conducted for each participant, to establish a regression line of heart rate against oxygen uptake. Calibrations took place ≥ 2 hours post prandially and after each participant had rested for 20-30 minutes upon arriving at the laboratory (Ceesay *et al.*, 1989; Livingstone, Robson & Totton, 2000; Moore *et al.*, 2004). The participants were also required to refrain from vigorous exercise on the day of the assessment. The heart rate calibration comprised of 4 minutes lying supine, 4 minutes sitting, 4 minutes standing, 4 minutes at level 1 of the netball fitness test (developed in chapter three), 4 minutes at level 2 of the netball fitness test, 4 minutes at level 3 of the netball fitness test and 4 minutes at level 4 of the netball fitness test. Details of the netball fitness test are provided in chapter three.

The FLEX heart rate calibration was then extended to a $\dot{V}O_{2\,peak}$ assessment in order to establish individual exercise intensities for each of the participants during the NSEP intervention in the present study. Following the final stage of the heart rate calibration the participants continued to complete the netball fitness test in order to identify each individual's $\dot{V}O_{2\,peak}$. Every 2 minutes the exercise intensity of the test was progressively increased from level 4 through to level 11. The criteria used to determine $\dot{V}O_{2\,peak}$ was based on those proposed by Armstrong and Welsman (2001) (see section

3.2.6). In chapter three of this thesis the netball fitness test was shown to be a meaningful test for assessing netball-specific endurance capability in 13-15 year old adolescent netball players. Although the $\dot{V}O_{2\,peak}$ values determined by the netball fitness test were lower compared to the multi-stage fitness test and the incremental treadmill test in chapter three of this thesis, the netball fitness test is a more ecologically valid assessment of netball fitness.

Heart rate and oxygen uptake (breath-by-breath then averaged over 60 seconds) were measured simultaneously during steady state exercise whilst completing the netball fitness test, with intensity increased every 4 minutes. Beat-by-beat heart rate, averaged per minute, was monitored by telemetry, whereby each participant was asked to wear a transmitter strap around their chest and a watch-like receiver on their wrist. Oxygen uptake was monitored using a facemask, attached via breathing tubes to a Metamax 3B portable gas analyser (Cortex, Leipzig, Germany). Both parameters were measured over the last 2 minutes of each level to ensure that values were stable.

In line with the methodology of Ceesay et al. (1989) a FLEX heart rate threshold value was established for each participant, as the mean of the highest heart rate during the standing measurement and the lowest heart rate during level 1 of the exercise.

5.2.5 Preliminary Measures: Day 2

Resting Metabolic Rate

Resting metabolic rate was assessed early in the morning, following a 12-hour period of fasting and no exercise. This measurement facilitated subsequent prediction of 24-hour energy expenditure. The procedure developed by Ventham and Reilly (1999) to assess resting metabolic rate in paediatric groups was used to assess resting metabolic rate, comprising a 5-10 minute rest period lying supine, followed by a 5-10 minute ‘settling in’ period and a subsequent 12-16 minute measurement period. The Quark b^2 breath-by-breath pulmonary gas exchange system (Quark b^2, Cosmed, Rome, Italy), with the canopy attachment was used to conduct the resting metabolic rate measurement.

5.2.6 Organisation of the Treatment Weeks

Treatment weeks A and B were organised as shown in figure 5.1.

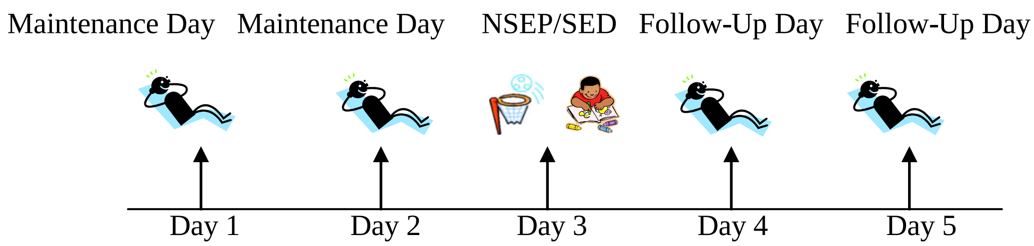


Figure 5.1. Organisation of treatment weeks.

Days 1 and 2 (Maintenance days)

For days 1 and 2 the participants were at school and these two days were deemed maintenance days where it was ensured that all the girls consumed a standardized diet and refrained from physical activity. This required the girls to consume the same foods and fluids prior to each treatment week. To ensure this was the case, combined weighed dietary records and 24-hour recalls were administered on each of the maintenance days during treatment week A and then the participants were asked to replicate their food and fluid intake on the maintenance days of treatment week B. Each participant was provided with a photocopy of their food diary and 24-hour recall interview which they completed on the maintenance days in treatment week A and were asked to replicate portion sizes and consume the same food and drink items on the maintenance days of treatment week B. Researchers liaised with parents to ensure this was the case. The purpose of this was to standardize energy intake before each treatment week, therefore all participants began each week at the same relative point to that of the previous week (Stubbs *et al.*, 2004).

With the aim of standardising energy expenditure on maintenance days 1 and 2, the researchers liaised with the head teacher and staff of the school as well as, parents to ensure regular curriculum activities were completed, whilst PE lessons and other 'active' classes such as dance and drama were avoided on these days. The participants were provided with a heart rate monitor and were asked to wear it during the waking hours of the maintenance days to assess energy expenditure. Each participant was provided with a VAS diary on day 1 during the preliminary measures familiarisation session. Therefore, hunger, fullness, prospective food consumption and mood were assessed using the VAS diary (see an example in appendix D), upon wakening each morning, before and after each meal and immediately before the participants went to bed each night.

Day 3 NSEP and SED Interventions

Day 3 was deemed the intervention day whereby the NSEP intervention was undertaken or SED activities were maintained. The participants were allowed breakfast at home on the morning of this day however they were asked to consume the same type and amount of food prior to each intervention, in order to standardize energy intake. At 08:15, the participants were collected from school by the researcher and transported to the university. At 09:00 half the group completed the NSEP and half were supervised whilst participating in sedentary activities for the duration of the 47 minute NSEP. Sedentary activities included reading or completing homework tasks. Minute-by-minute heart rate was monitored throughout both interventions. Following the completion of the NSEP the participants were returned to school and remained sedentary for the rest of the day.

The NSEP was based on a netball specific fitness test developed by Gasston and Simpson (2004). Gasston and Simpson (2004) identified through match analysis movements inherent in a netball match (walking forwards and backwards, jogging forwards and backwards, running forwards and backwards, turning, jumping, lunging, sidestepping, foot-specific agility and a choice reaction task), the majority of which are included in the NSEP. The NSEP was designed so that set exercise intensities were not imposed on the participants. Instead it was a self-paced session in which the girls decided how hard they worked, as is the case during a netball match, since player position determines work rate (Otago, 1983). The participants were told to work as per a regular netball match.

The 47 minute NSEP was organised in the same way as a netball match. The participants exercised in four 10 minute bouts each representing one quarter of a netball match, followed by a 2 or 3 minute rest interval (figure 5.2). During this rest period the participants remained standing.

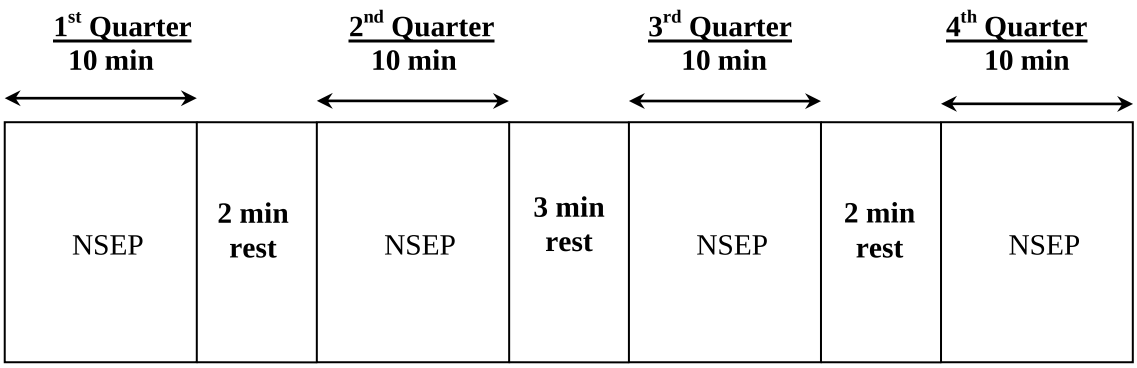
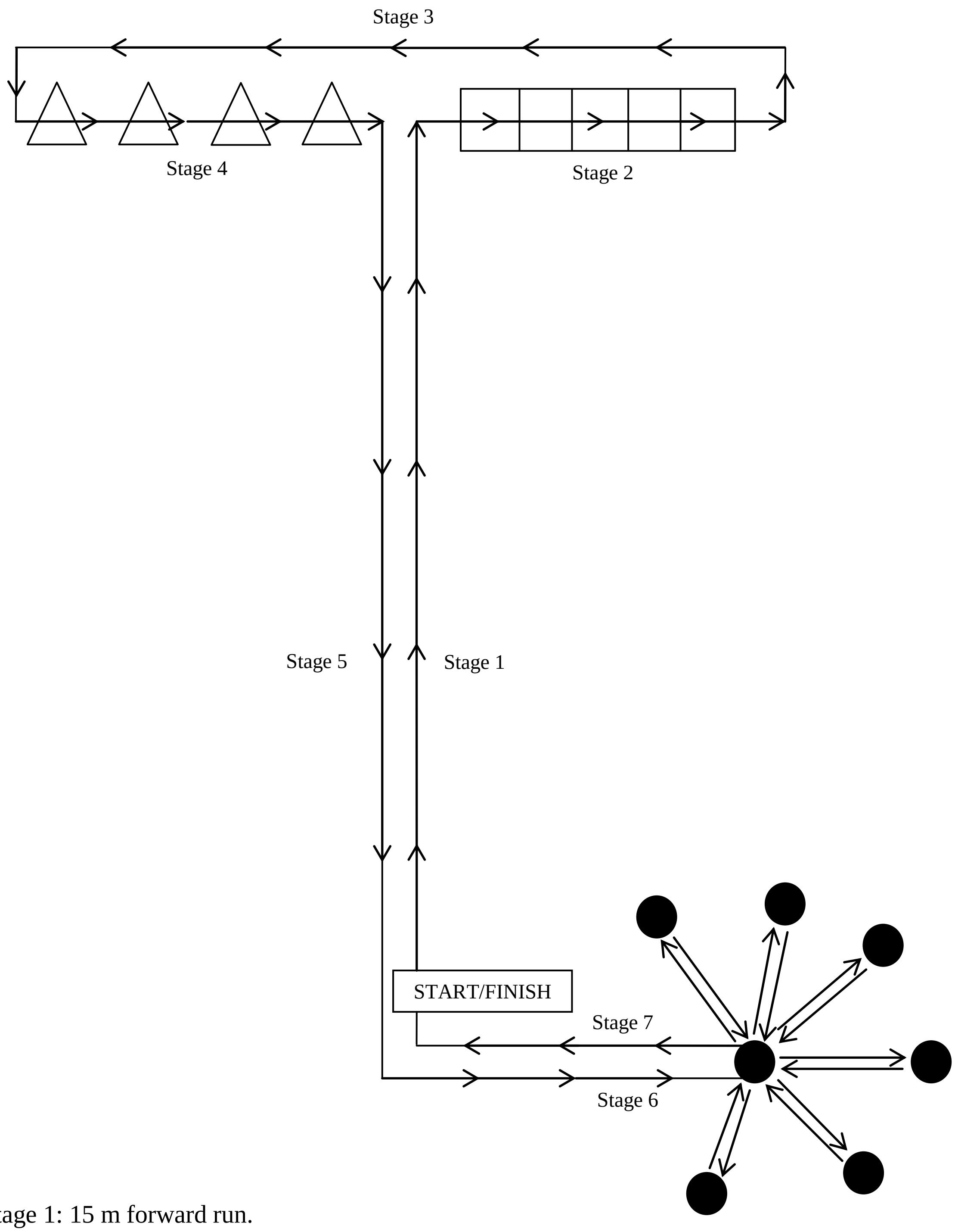


Figure 5.2. Netball specific exercise protocol (NSEP) organisation.

Each NSEP quarter consisted of seven stages in the form of a circuit (figure 5.3) with a time limit for completion of 15 seconds. Netball players are generally involved in work periods of 10 seconds and less during a netball match (Otago, 1983), but no longer than 15 seconds before a recovery period is available (Allison, 1978). Therefore after completing the exercise in each stage, the participants were asked to remain stationary until the full 15 seconds for that stage had elapsed. This ensured varying work to rest ratios of between 1:2 and 1:3 or greater (Otago, 1983) were maintained. This is similar to that of a netball match.

The Pictorial Children's Effort Rating Table (Yelling, Lamb & Swaine, 2002) was administered before the NSEP and during each rest interval and heart rate was recorded simultaneously with the Pictorial Children's Effort Rating Table to provide an indication of perceived exertion. The scale ranged from 1 to 10, 1 depicting the exercise as being *very, very easy* and 10 as being *so hard I'm going to stop*

Hunger, prospective food consumption, fullness and mood were assessed immediately before and after the NSEP and SED interventions, using the VAS diary.



Stage 1: 15 m forward run.
Stage 2: 5 m agility ladder.
Stage 3: 10 m side stepping.
Stage 4: two footed jumps over four 30 cm hurdles, covering 5 m.
Stage 5: 15 m backward run.
Stage 6: 5 m forward run, lunge towards 1 cone (each 3 m from centre spot) and return to centre spot, repeat this three times then finish at centre spot.
Stage 7: 5 m forward walk.

Figure 5.3. Details of one circuit of the netball specific exercise protocol (NSEP).

Days 4 and 5 (Follow-up days)

Days 4 and 5 were deemed follow-up days, where the participants attended school. During these days the participants were asked to continue with their normal daily food and fluid intake and curriculum activities, but to avoid PE lessons and classes such as dance rehearsals and drama. They were encouraged not to participate in activities which would elevate their heart rate, such as running and cycling, in and out of school time. The researcher liaised with staff at the school and also parents to facilitate this. Heart rate and energy intake were measured during the waking hours of each day. The participants were provided with a VAS booklet and were asked to record their hunger, prospective food consumption, fullness and mood on each of these days, upon wakening, before and after each meal and immediately before going to bed each night.

5.2.7 Heart Rate Monitoring

Minute-by-minute heart rate was recorded using telemetry during the waking hours of the study days, from 09:00 on day 1 through to 09:00 on day 6. During sleeping periods the participants were not required to wear the heart rate monitors and receivers, as it was demonstrated in a pilot study that the participants found wearing the heart rate monitors and receivers at night uncomfortable. Data was retrieved at approximately 15:00 on each of the study days, when the girls had finished school, using a Polar USB 2.0 IrDA IRwave Infrared Adapter, for use with the Polar RS-400 and a Polar Infrared Interface for use with the Polar S610i. Heart rate monitors were swapped with a replacement monitor each day to maintain data collection.

5.2.8 Estimation of Energy Expenditure

Energy expenditure was estimated assuming 20.5 kJ/L of oxygen consumed per minute (Weir, 1949). Energy expenditure for periods when heart rate fell below the FLEX heart rate threshold value was calculated as the equivalent to the mean of the resting activities (lying supine, sitting and standing) (Ceesay *et al.*, 1989) and was referred to as the resting energy expenditure. When heart rate values were above the FLEX heart rate threshold value, energy expenditure was derived from the minute-by-minute recorded heart rate by reference to the individual's heart rate and oxygen uptake regression line and referred to as the activity energy expenditure. When unphysiological high pulse rates (>220 $b \cdot min^{-1}$, which indicated interference) and zero values were identified, these values were removed and replaced by the average of the previous and subsequent values (Wareham *et al*., 1997). Energy expenditure during sleep was derived from resting metabolic rate data.

Subsequently, 24-hour energy expenditure was estimated by summing resting energy expenditure, activity energy expenditure and resting metabolic rate (Ceesay *et al.*, 1989; Livingstone, Robson & Totton, 2000) for each participant.

5.2.9 Estimation of Energy Intake

Energy intake was estimated using a combined self-reported, weighed food diary and 24-hour recall interview technique (chapter four) from 09:00 on day 1 through to 09:00 on day 6. The face-to-face 24-hour food recalls took place on the school premises at approximately 15:00 each day since this was the end of the school day. The participants were also asked to take responsibility for the measurement of their own food and fluid intake. To facilitate the recording process each participant was issued with a set of dietary scales (Gormet White Electronic Scales, Hanson) and a food diary (an example of the content is provided in appendix C) which included a set of written instructions. Participants were not required to estimate food portions away from the home as this has thought to interfere with usual eating behaviour (Livingstone *et al.*, 1992b). Instead researchers liaised with the kitchen staff at the school and also where feasible foods were purchased to obtain the required nutritional information from food packaging. If the participants did not identify food portions, amounts were substituted with previous values reported by the participant or using standard portion estimates from the Dietmaster Software Package [Dietmaster (1999) Version 4.0 Professional Edition, Swift Computer Systems].

In terms of estimating total daily energy intake a hierarchical method was employed (Dodd, Welsman & Armstrong, 2008). To obtain nutritional information for each food item, the recipes for school dinners and lunch items were obtained from the Scolarest handbooks [Scolarest (2005) Compass Group UK & Ireland Limited], the company who supplied the school with food and beverages. Secondly and where possible, information was gathered using the Tesco supermarket website (www.tesco.co.uk) or by visiting supermarkets in person to study the actual food packaging. Otherwise, the Dietmaster Software Package [Dietmaster (1999) Version 4.0 Professional Edition, Swift Computer Systems] was used for all other remaining foods that required nutritional information; and if the food item could not be identified using this database, information was gathered using a text book by McCance and Widdowson (Holland *et al.*, 2001) which outlines the compostion of foods.

5.2.10 Subjective Appetite

Appetite (hunger, prospective food consumption and fullness) parameters were assessed from 09:00 hours on day 1 until 09:00 hours on day 6. The VAS were used to monitor subjective feelings of hunger, fullness and prospective food consumption in the form of 100 mm horizontal lines (Hill & Blundell, 1982; Flint *et al.,* 2000). The VAS required the participants to answer the following questions, 'How hungry do you feel now?' which was anchored by *very hungry (100)* and *not at all hungry (0),* 'How full do you feel now?' which was anchored by *very full (100)* and *not full at all (0)*; prospective food consumption, "How much would you like to eat now?" which were anchored by *a lot (100)* and *nothing at all (0).* The participants were required to mark a vertical line on the VAS to correspond with what they were subjectively feeling at the time the VAS were administered. The VAS were administered immediately before and after each 47 minute intervention period (NSEP and SED) on day 3, upon wakening on all days, before and after each meal on all days and immediately before going to bed each night.

5.2.11 Mood

Mood parameters were also assessed from 09:00 on day 1 until 09:00 on day 6. The VAS were used to monitor mood states on 100 mm horizontal lines. The parameters alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood were assessed, adapted from Bond and Lader (1974). These were administered at exactly the same time points as the VAS for appetite.

5.2.12 Exercise Intensity, Ratings of Perceived Exertion and Heart Rate

For the 47 minute NSEP intervention, mean ± SD exercise intensity as a percentage of $\dot{V}O_{2\ peak}$ and as a percentage of maximum heart rate was calculated. This was done by calculating each individuals mean $\dot{V}O_2$ and heart rate during the exercise periods of the NSEP and determining what percentage this was of their $\dot{V}O_{2\ peak}$ and maximum heart rate. These values were then averaged to provide an overall indication of exercise intensity in terms of $\dot{V}O_{2\ peak}$ and percentage of maximum heart rate induced by the NSEP. Mean ± SD were also calculated for ratings of perceived exertion using the Pictorial Children's Effort Rating Table and the corresponding heart rate ($b \cdot min^{-1}$), immediately before the NSEP intervention, after every 10 minute quarter during the NSEP and immediately after the NSEP intervention.

5.2.13 Retrospective Questionnaire

A retrospective questionnaire (see appendix E) was developed with the intention of providing a brief evaluation of the study protocol. This was administered after completion of the full study protocol, to provide an indication of how the girls felt about aspects of the study, in order to inform subsequent studies of this nature. These included questions associated with areas of the study they found the most and least enjoyable, similarity of the NSEP to a netball match, how the girls felt remaining inactive for the treatment weeks and if they felt they altered their food and drink intake due to the food diary they were completing.

5.2.14 Statistical Analysis

The statistical package SPSS-PC (SPSS Inc., Chicago, IL) was used for all data analyses. Prior to analysis all data was assessed for normality using the Shapiro-Wilks' test. Means ± SD were calculated for all data.

Days 1 and 2 (Maintenance Days)

Mean daily 24-hour energy balance ($MJ \cdot d^{-1}$) was calculated by subtracting 24-hour energy expenditure ($MJ \cdot d^{-1}$) from 24-hour energy intake ($MJ \cdot d^{-1}$) for days 1 and 2. Mean daily 24-hour energy intake ($MJ \cdot d^{-1}$), 24-hour energy expenditure ($MJ \cdot d^{-1}$), and 24-hour energy balance ($MJ \cdot d^{-1}$) were analysed for main effects of intervention or maintenance days and interactions between interventions and maintenance days using a 2 (intervention: NSEP v SED) x 2 (maintenance day) ANOVA (within-within design).

For each macronutrient (percentage of daily energy intake), protein, carbohydrate and fat, for the two maintenance days a 2 (intervention: NSEP v SED) x 2 (maintenance day) ANOVA (within-within design) was used to identify any main effects of intervention or maintenance days and interactions between interventions and maintenance days.

For each of the appetite parameters an average of all the time points sampled (immediately upon waking each day, before meal, after meal and finally immediately before bed each night) for each of the maintenance days, in both the NSEP and SED treatment weeks was calculated, to provide a mean total daily hunger, prospective food consumption and fullness rating. Hunger, prospective food consumption and fullness were then analysed individually for main effects and interactions using a 2 (intervention: NSEP v SED) x 2 (maintenance day) ANOVA (within-within design).

All of the mood parameters, alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood were analysed individually, in the same way as the appetite parameters.

Day 3 NSEP and SED Interventions

Mean ± SD energy expenditure for the 47 minute NSEP and SED intervention periods was calculated and analysed using a one way repeated measures ANOVA. Following the 47 minute NSEP and SED intervention period, mean ± SD time of eating onset was calculated. This was analysed using a one way repeated measures ANOVA.

Mean values for hunger, prospective food consumption, fullness were calculated for immediately before the NSEP intervention and immediately after the NSEP intervention on day 3. Such calculations were also conducted for the corresponding SED intervention. With regards to exercise and subjective appetite, the main area of interest was before versus following the NSEP and also subjective appetite immediately following both the NSEP and SED interventions. Therefore, more specifically, a 2 (time: pre v post) x 2 (intervention: NSEP v SED) ANOVA (within-within design) was conducted for each of the appetite parameters, hunger, prospective food consumption and fullness.

All of the mood parameters (alertness, activeness, tiredness, happiness, boredom, relation and overall mood) were analysed in the same way as the appetite parameters.

Ratings of perceived exertion measured using the Pictorial Children's Effort Rating Table and heart rate, immediately before the NSEP, 10 minutes, 20 minutes and 30 minutes during the NSEP and immediately after the NSEP were analysed using a one way repeated measures ANOVA.

Days 3, 4 and 5 (Intervention day and follow-up days)

Mean daily 24-hour energy intake ($MJ \cdot d^{-1}$), 24-hour energy expenditure ($MJ \cdot d^{-1}$) and 24-hour energy balance ($MJ \cdot d^{-1}$) were analysed for main effects of intervention or week day and interactions between interventions and week days, using a 3 (week day) x 2 (intervention: NSEP v SED) ANOVA (within-within design). Further post-hoc analyses included repeated measures ANOVA and Tukey tests as appropriate. For day 3 and day 4, 24-hour energy intakes ($MJ \cdot d^{-1}$) were combined to provide a value for 2-day (48-hour) energy intake (MJ). This was also the case for day 3 and day 4 24-hour energy expenditure ($MJ \cdot d^{-1}$) and 24-hour energy balance ($MJ \cdot d^{-1}$). Similarly for day 3, day 4 and day 5, 24-hour energy intakes ($MJ \cdot d^{-1}$) were combined to provide a value for 3-day (72-hour) energy intake (MJ). This was also the case for day 3, day 4 and day 5, 24-hour energy expenditure

($MJ \cdot d^{-1}$) and for day 3, day 4 and day 5 and energy balance ($MJ \cdot d^{-1}$). Subsequently, 2-day energy intake (MJ) and 2-day energy balance (MJ) and 3-day energy intake (MJ), 3-day energy expenditure (MJ) and 3-day energy balance (MJ) were analysed using a one way repeated measures ANOVA.

For each macronutrient (percentage of daily energy intake), protein, carbohydrate and fat, for day 3, 4 and 5 a 2 (intervention: NSEP v SED) x 3 (week day) ANOVA (within-within design) was used to identify any main effects of intervention or week day and interactions between interventions and week days.

For each of the appetite parameters an average of each of the time points sampled during day 3, 4 and 5 (immediately upon waking each day, before meal, after meal and finally immediately before bed each night) in both the NSEP and SED interventions was calculated, to provide a mean daily hunger, prospective food consumption and fullness rating. These were then analysed for main effects and interactions using a 3 (week day) x 2 (intervention: NSEP v SED) ANOVA (within-within design). Further post-hoc analyses included repeated measures ANOVA and Tukey tests as appropriate.

All of the mood parameters (alertness, activeness, tiredness, happiness, boredom, relation and overall mood) were analysed in the same way as the appetite parameters.

Retrospective Questionnaire

The main question of interest from the retrospective questionnaire with regards to this study was "*did the NSEP feel like you were playing netball? If yes, why? If no, why?*" The percentage of participants who agreed ('yes') and disagreed ('no') with this question was calculated.

For all one way repeated measures ANOVA a Mauchly's sphericity test was conducted and the Greenhouse-Geisser correction was applied if the assumption of sphericity had been violated. When significant differences had been identified, Cohen's d effect size for one-way ANOVA was calculated and interpreted against the effect size categories of ≤ 0.1 = small effect, ~ 0.25 = moderate effect and ≥ 0.4 = large effect (Cohen, 1988; 1992). The significance level was set at $p<0.05$ for all analyses.

5.3 Results

The age and physical characteristics (mean ± SD) of the participants were: age 14.7±0.9 years old; stature 1.66±0.06 m; mass 59.4±7.1 kg; body fat 23.1±5.4% and BMI 21.6±2.3 kg/m^2, thus all classified as being normal weight according to Cole et al. (2000).

5.3.1 Energy Expenditure

Mean ± SD daily 24-hour energy expenditure for each day in each treatment week is provided in figure 5.4.

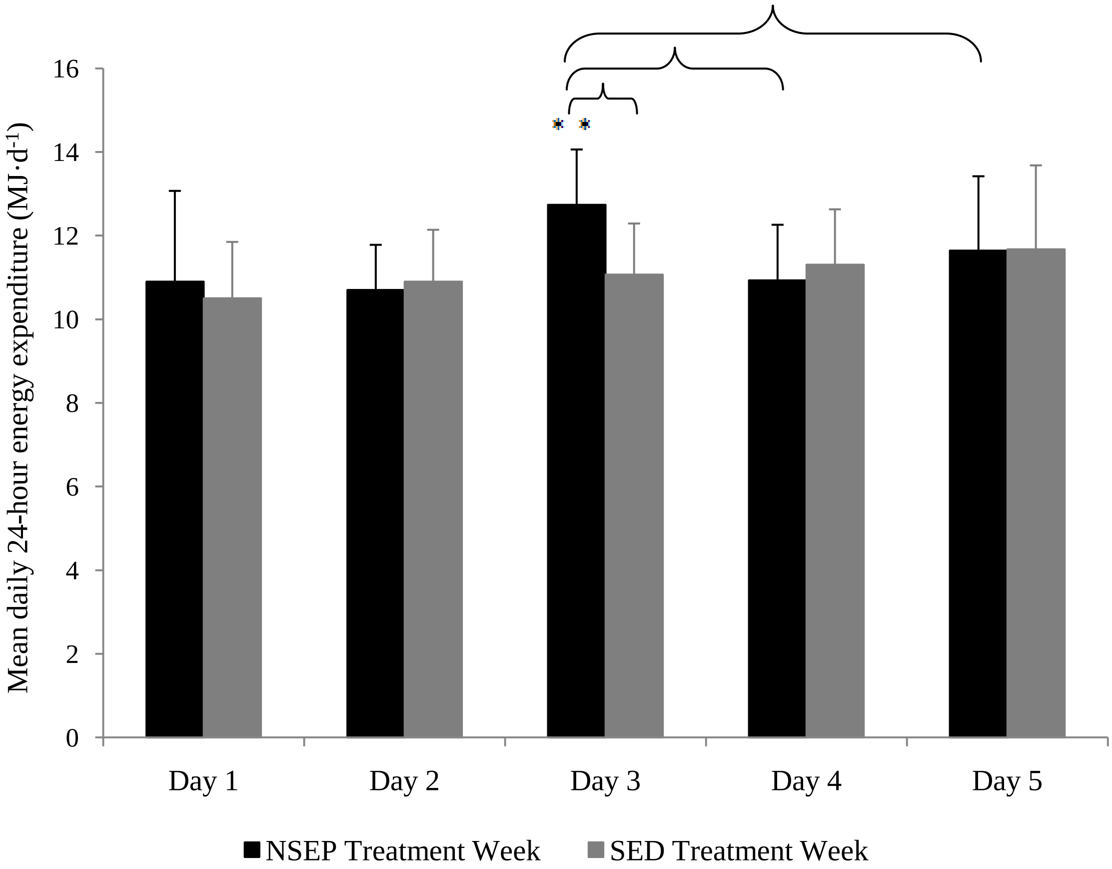


Figure 5.4. Mean ± SD daily 24-hour energy expenditure (MJ) for all participants (n=11), for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. **Significantly elevated between treatment weeks (*p*=0.002) and compared to day 4 (*p*<0.01) and day 5 (*p*<0.05) in the NSEP treatment week.

Days 1 and 2 (Maintenance Days)

For daily 24-hour energy expenditure for the maintenance days 1 and 2, there was no significant main effect of intervention [$F(1,10)=0.108$, $p=0.750$] or day [$F(1,10)=0.127$, $p=0.728$] or interaction effect between intervention and day [$F(1,10)=0.966$, $p=0.349$].

Day 3 NSEP and SED Interventions

Average exercise-induced energy expenditure was significantly higher for the 47 minute NSEP intervention compared to the 47 minute SED intervention 1.44±0.18 and 0.33±0.04 MJ, respectively [$F(1,10)=385.258$, $p<0.001$, effect size = 8.78].

Exercise Intensity of the NSEP

The self-paced NSEP induced an average exercise intensity of 64±5% of $\dot{V}O_{2\ peak}$, with the girls working on average at 83±6% of maximum heart rate.

Ratings of Perceived Exertion and Heart Rate during the NSEP

Mean ± SD ratings of perceived exertion values (using the Pictorial Children's Effort Rating Table) and heart rate sampled immediately before and after the NSEP intervention and after each 10 minute quarter during the NSEP intervention, are provided in table 5.2.

Table 5.2. Mean ± SD ratings of perceived exertion using the Pictorial Children's Effort Rating Table (P-CERT) and heart rate (b·min^{-1}), for all participants (n=11), before, during and after the netball specific exercise protocol (NSEP) intervention.

	P-CERT		**Heart rate (b·min^{-1})**	
	Mean	**SD**	**Mean**	**SD**
Immediately before NSEP	1	0*	97	12†
10 minutes into NSEP	4	1**	164	15
20 minutes into NSEP	5	1***	171	15
30 minutes into NSEP	6	2	170	12
Immediately after NSEP	6	1	169	18

***Significantly lower compared to 10 minutes, 20 minutes, 30 minutes and immediately after the NSEP intervention (*p*<0.01). **Significantly lower compared to 20 minutes into, 30 minutes into and immediately after the NSEP intervention (*p*<0.01). *** Significantly lower compared to immediately after the NSEP intervention (*p*<0.01). † Significantly lower compared to 10 minutes into, 20 minutes into, 30 minutes into and immediately after the NSEP intervention (*p*<0.01).**

For ratings of perceived exertion using the Pictorial Children's Effort Rating Table, there was a significant main effect of time [$F(1.467,14.673)=88.063$, $p<0.001$]. Post-hoc Tukey tests identified that ratings of perceived exertion values immediately before the NSEP intervention were significantly lower ($p<0.01$) compared to ratings of perceived exertion values 10 minutes into, 20 minutes into, 30 minutes into and immediately after the NSEP intervention. Ratings of perceived exertion values 10 minutes into the NSEP intervention ($p<0.01$) were significantly lower compared to 20 minutes into, 30 minutes into and immediately after the NSEP intervention. Ratings of perceived exertion values 20 minutes into the NSEP intervention were significantly lower ($p<0.01$) compared to immediately after the NSEP intervention.

For heart rate there was also a significant main effect of time [$F(1.754, 17.353)=142.441$, $p<0.001$]. Post-hoc Tukey tests confirmed that heart rate values immediately before the NSEP intervention were significantly lower ($p<0.01$) compared to 10 minutes into, 20 minutes into, 30 minutes into and immediately after the NSEP intervention.

Days 3, 4 and 5 (Intervention day and follow-up days)

For day 3, 4 and 5 daily 24-hour energy expenditure, significant main effects were found for week day [$F(2,20)=4.244$, $p=0.029$] but not for intervention [$F(1,10)=1.149$, $p=0.309$]. A significant interaction was found for intervention by week day [$F(2,20)=6.356$, $p=0.007$]. Further one way repeated measures ANOVA identified that energy expenditure was elevated on day 3 in the NSEP treatment week when compared to the corresponding day in the SED treatment week, 12.74±1.32 versus 11.07±1.22 $MJ \cdot d^{-1}$, respectively [$F(1,10)=16.229$, $p=0.002$, effect size = 1.8]. Post-hoc Tukey tests also confirmed an elevated energy expenditure for day 3 when compared to day 4 ($p<0.01$) and day 5 ($p<0.05$) within the NSEP treatment week, 12.74±1.32 versus 10.93±1.33 and 11.64±1.78 $MJ \cdot d^{-1}$, respectively. For 72-hour energy expenditure (sum of day 3, 4 and 5) when the NSEP treatment week was compared to the SED treatment week, there was no significant main effect found for condition, 35.32±4.00 versus 34.05±3.82 MJ, respectively [$F(1,10)=1.149$, $p=0.309$].

5.3.2 Energy Intake

Mean ± SD daily 24-hour energy intake for each day in each treatment week is provided in figure 5.5.

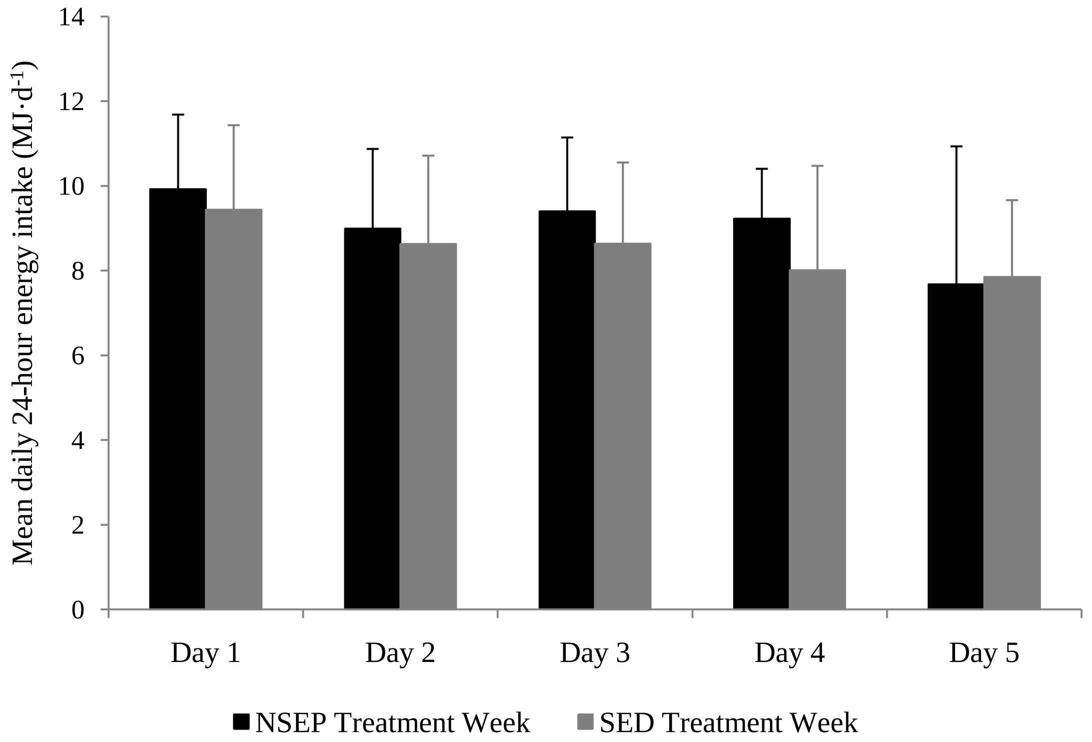


Figure 5.5. Mean ± SD daily 24-hour energy intake (MJ) for all participants (n=11), for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

Days 1 and 2 (Maintenance days)

For daily 24-hour energy intake for the maintenance days 1 and 2, there was no significant main effect of intervention [$F(1,10)=1.002$, $p=0.340$] or day [$F(1,10)=1.856$, $p=0.203$] or interaction effect between intervention and day [$F(1,10)=0.072$, $p=0.794$].

Day 3 NSEP and SED Interventions

Following the 47 minute NSEP and SED intervention period, mean ± SD time of eating onset between interventions was not significantly different [$F(1,10)=0.413$, $p=0.535$], 2:13±0.16 and 1:59±0.05 hours:mins, respectively.

Days 3, 4 and 5 (Intervention day and follow-up days)

For day 3, 4 and 5 daily 24-hour energy intake, there were no significant main effects of intervention [$F(1,10)=2.143$, $p=0.174$] or week day [$F(2,20)=2.289$, $p=0.127$] or interaction effect between intervention and week day [$F(2,20)=1.022$, $p=0.378$].

Figure 5.6 shows that 2-day (48-hour) energy intake was significantly higher for the NSEP treatment week compared to the corresponding days in the SED week, 18.63±2.49 versus 16.65±3.48 MJ respectively [$F(1,10)=8.611$, $p=0.015$, effect size =

1.31]. This was not apparent for 3-day (72-hour) energy intake between treatment weeks [*F*(1,10)=2.143, *p*=0.174] (figure 5.6).

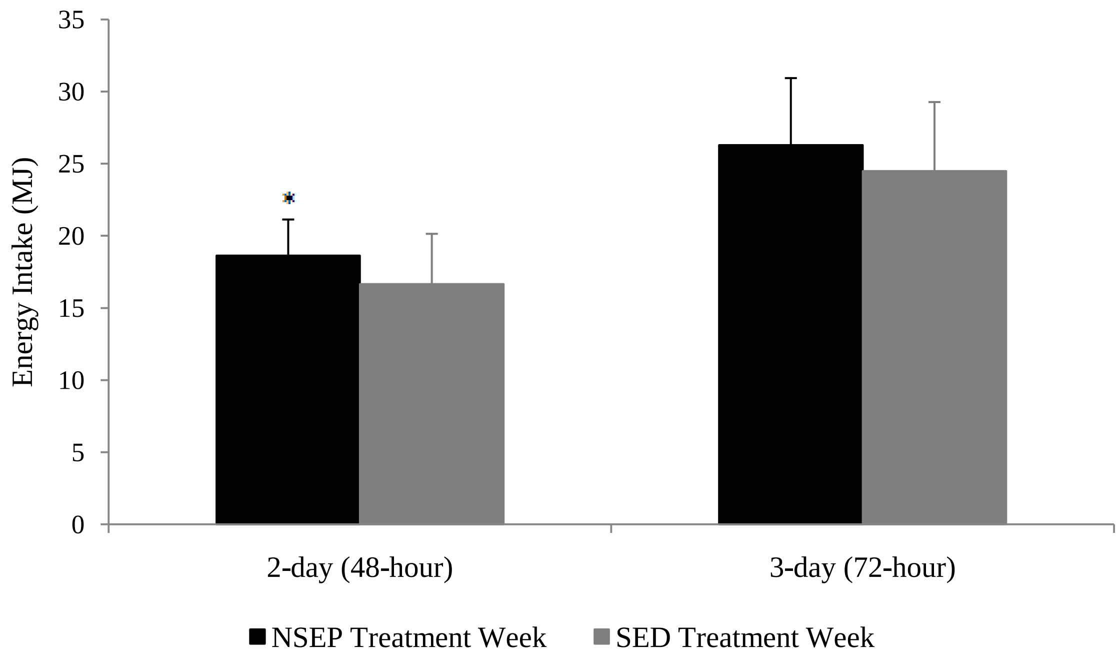


Figure 5.6. Mean ± SD 2-day (48-hour) and 3-day (72-hour) energy intakes (MJ) for all participants (n=11), in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. ***2-day (48-hour) energy intake (MJ) was significantly higher in the NSEP treatment week compared to the SED treatment week (*p*=0.015).**

5.3.3 Macronutrient Intake

Mean ± SD percentage of daily energy intake for all three macronutrients, protein, carbohydrate and fat, over days 1 to 5 in each treatment week are provided in figure 5.7.

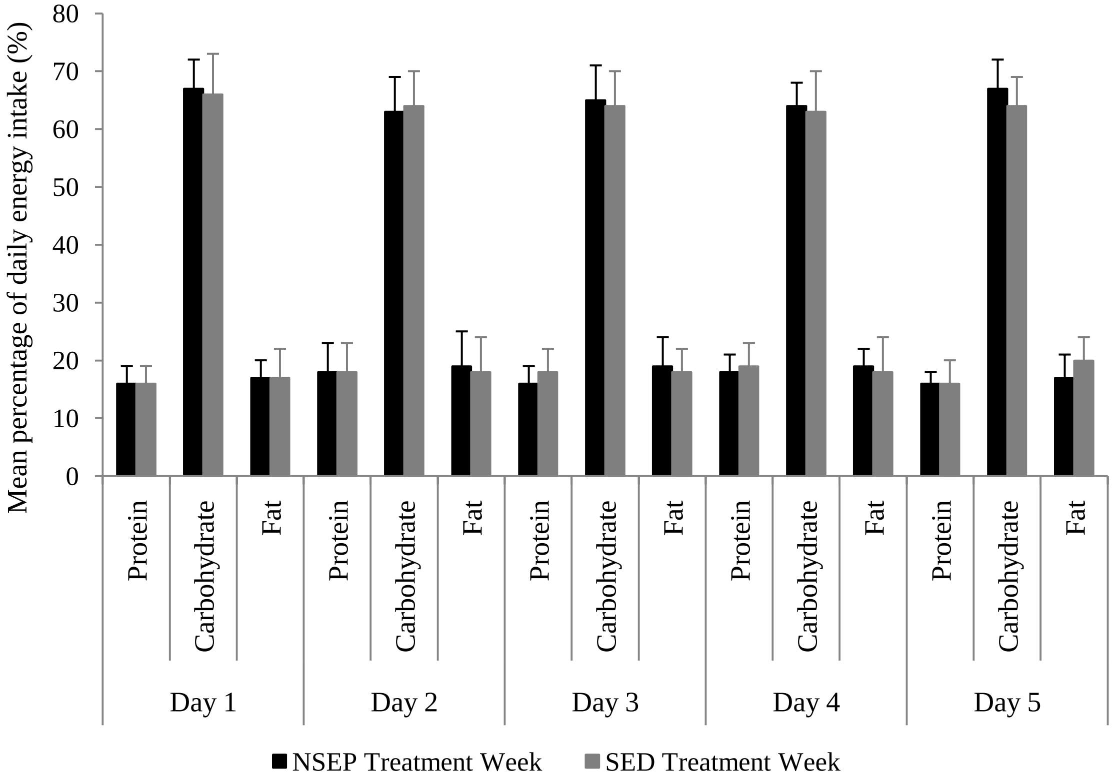


Figure 5.7. Mean ± SD macronutrient intakes as a percentage of daily energy intake, for all participants (n=11) for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

Days 1 and 2 (Maintenance days)

There was no main effect of intervention for protein [$F(1,10)=0.828$, $p=0.384$], carbohydrate [$F(1,10)=0.127$, $p=0.729$] or fat [$F(1,10)=0.286$, $p=0.604$] or main effect of week day for protein [$F(1,10)=1.878$, $p=0.201$], carbohydrate [$F(1,10)=3.077$, $p=0.110$] or fat [$F(1,10)=0.727$, $p=0.414$]. Similarily, there was no interaction effect between intervention and week day for the maintenance days 1 and 2, for protein [$F(1,10)=0.101$, $p=0.757$], carbohydrate [$F(1,10)=0.302$, $p=0.595$] or fat [$F(1,10)=0.192$, $p=0.671$].

Days 3, 4 and 5 (Intervention day and follow-up days)

Similarly, for day 3, 4 and 5, there was no main effect of intervention for protein [$F(1,10)=1.535$, $p=0.244$], carbohydrate [$F(1,10)=0.880$, $p=0.370$] or fat [$F(1,10)=0.040$, $p=0.845$] or main effect of week day for protein [$F(2,20)=1.835$, $p=0.186$], carbohydrate [$F(2,20)=0.920$, $p=0.415$] or fat [$F(2,20)=0.093$, $p=0.911$]. Similarily, there was no interaction effect between intervention and week day for days 3, 4 and 5, for protein [$F(2,20)=0.625$, $p=0.545$], carbohydrate [$F(2,20)=0.422$, $p=0.662$] or fat [$F(2,20)=2.350$, $p=0.121$].

5.3.4 Energy Balance

Mean ± SD daily 24-hour energy balance (energy intake – energy expenditure) for each day in each treatment week is provided in table 5.3.

Table 5.3. Mean ± SD daily 24-hour energy balance ($MJ \cdot d^{-1}$), for all participants (n=11), for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

	NSEP		SED	
	Mean	**SD**	**Mean**	**SD**
Day 1 ($MJ \cdot d^{-1}$)	-0.98	2.93	-1.06	2.52
Day 2 ($MJ \cdot d^{-1}$)	-1.71	2.25	-2.27	2.69
Day 3 ($MJ \cdot d^{-1}$)	-3.34	2.46	-2.43	2.10
Day 4 ($MJ \cdot d^{-1}$)	-1.71	1.89	-3.31	2.70
Day 5 ($MJ \cdot d^{-1}$)	-3.44	3.33	-3.82	3.09
Day 3 + Day 4 (2-day, 48-hour) (MJ)	-5.05	3.72	-5.73	4.04
Day 3 + Day 4 + Day 5 (3-day, 72-hour) (MJ)	-8.49	6.21	-9.55	6.57

Energy balance was calculated as energy intake – energy expenditure.

Days 1 and 2 (Maintenance days)

For daily 24-hour energy balance for the maintenance days 1 and 2, there was no significant main effect of intervention [$F(1,10)=0.255$, $p=0.624$] or day [$F(1,10)=2.370$, $p=0.155$] or interaction effect between intervention and day [$F(1,10)=0.376$, $p=0.553$].

Days 3, 4 and 5 (Intervention day and follow-up days)

For day 3, 4 and 5 daily 24-hour energy balance, there were no significant main effects of intervention [$F(1,10)=0.823$, $p=0.386$] or week day [$F(2,20)=1.740$, $p=0.201$] or interaction effect between intervention and week day [$F(2,20)=2.702$, $p=0.091$].

For 48-hour and 72-hour energy balance, there was no difference between the NSEP and SED treatment weeks [$F(1,10)=0.464$, $p=0.511$] and [$F(1,10)=0.823$, $p=0.386$], respectively.

5.3.5 Subjective Appetite

Mean ± SD daily hunger, prospective food consumption and fullness ratings for days 1 to 5 are presented in table 5.4.

Table 5.4. Mean ± SD appetite ratings for hunger, prospective food consumption and fullness (mm), for all participants (n=11) for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

	Hunger (very hungry = 100mm)				**Prospective food consumption (a lot = 100mm)**				**Fullness (very full = 100mm)**			
	NSEP Mean	**SD**	**SED Mean**	**SD**	**NSEP Mean**	**SD**	**SED Mean**	**SD**	**NSEP Mean**	**SD**	**SED Mean**	**SD**
Day 1	40	7	44	16	37	7	42	16	47	9	47	18
Day 2	45	11	47	11	43	10	45	13	52	10	52	11
Day 3	46	12	45	10	43	13	44	11	52	11	49	8
Day 4	46	10	44	10	46	11	44	10	52	7	49	9
Day 5	46	11	45	9	46	12	44	10	53	10	50	7

Days 1 and 2 (Maintenance days)

There was no significant main effect of intervention for hunger [$F(1,10)=0.616$, $p=0.451$], prospective food consumption [$F(1,10)=0.337$, $p=0.574$], or fullness [$F(1,10)=0.000$, $p=0.987$] or main effect of week day for hunger [$F(1,10)=4.391$, $p=0.063$], prospective food consumption [$F(1,10)=3.745$, $p=0.082$], or fullness [$F(1,10)=2.340$, $p=0.157$]. Similarily there was no interaction effect between intervention and week day for hunger [$F(1,10)=0.919$, $p=0.360$], prospective food consumption [$F(1,10)=1.020$, $p=0.336$] and fullness [$F(1,10)=0.030$, $p=0.867$].

Day 3 NSEP and SED Interventions

For hunger only, it was identified that the girls felt significantly more hungry immediately following the NSEP intervention compared to immediately before [$F(1,10)=5.790$, $p=0.037$, effect size = 1.08], 49±25 and 35±21 mm, respectively.

Days 3, 4 and 5 (Intervention day and follow-up days)

There were no significant main effects of intervention for hunger [$F(1,10)=0.729$, $p=0.413$], prospective food consumption [$F(1,10)=0.790$, $p=0.395$], or fullness [$F(1,10)=1.829$, $p=0.206$] or main effect of week day for hunger [$F(2,20)=0.086$, $p=0.918$], prospective food consumption [$F(2,20)=0.317$, $p=0.732$], or fullness [$F(2,20)=0.417$, $p=0.665$]. Similarily, there was no interaction effect between intervention and week day for hunger [$F(2,20)=0.152$, $p=0.860$], prospective food consumption [$F(2,20)=0.768$, $p=0.477$] and fullness [$F(2,20)=0.002$, $p=0.998$] for days 3, 4 and 5.

5.3.6 Mood

Days 1 and 2 (Maintenance days)

For daily alertness ratings for the maintenance days 1 and 2, there was no significant effect of intervention [$F(1,10)=0.973$, $p=0.347$] or week day [$F(1,10)=0.591$, $p=0.460$], however there was an interaction effect between intervention and day [$F(1,10)=10.008$, $p=0.010$]. Further one way repeated measures ANOVA identified that the girls felt more alert on day 1 in the SED treatment week when compared to the corresponding day in the NSEP treatment week, 60±8 mm versus 53±10 mm, respectively [$F(1,10)=7.516$, $p=0.021$, effect size = 1.23].

For daily activeness ratings for the maintenance days 1 and 2, there was no significant effect of intervention [$F(1,10)=0.005$, $p=0.944$] or week day [$F(1,10)=3.072$, $p=0.110$] however there was an interaction effect between intervention and day [$F(1,10)=17.012$, $p=0.002$]. Post-hoc Tukey tests confirmed that the girls felt more active on day 2 compared to day 1 within the NSEP treatment week, 56±14 mm versus 46±11 mm, respectively ($p<0.05$).

Day 3 NSEP and SED Interventions

It was identified that the girls felt significantly more alert immediately following the NSEP intervention compared to immediately after the SED intervention [$F(1,10)=6.620$, $p=0.028$, effect size 1.1], 66±15 mm versus 60±16 mm, respectively.

Days 3, 4 and 5 (Intervention day and follow-up days)

For day 3, 4 and 5, there were no significant main effects of intervention for daily alertness [$F(1,10)=0.114$, $p=0.743$], activeness [$F(1,10)=0.009$, $p=0.928$], tiredness [$F(1,10)=1.590$, $p=0.236$], happiness [$F(1,10)=0.544$, $p=0.478$], boredom [$F(1,10)=0.003$, $p=0.961$], relaxation [$F(1,10)=2.836$, $p=0.123$] and overall mood [$F(1,10)=0.702$, $p=0.422$]. There were also no main effects of week day for daily alertness [$F(2,20)=2.561$, $p=0.102$], activeness [$F(2,20)=0.662$, $p=0.527$], tiredness [$F(2,20)=1.308$, $p=0.293$], happiness [$F(2,20)=1.049$, $p=0.369$], boredom [$F(2,20)=0.299$, $p=0.745$], relaxation [$F(2,20)=0.714$, $p=0.502$] and overall mood [$F(2,20)=0.342$, $p=0.714$]. Similarily, there were no interaction effects between intervention and week day for daily alertness [$F(2,20)=0.632$, $p=0.542$], activeness [$F(2,20)=1.554$, $p=0.236$], tiredness [$F(2,20)=0.088$, $p=0.916$], happiness [$F(2,20)=0.942$, $p=0.406$], boredom [$F(2,20)=1.155$, $p=0.335$], relaxation [$F(2,20)=0.851$, $p=0.442$] and overall mood [$F(2,20)=0.318$, $p=0.731$].

5.3.7 Retrospective Questionnaire

Responses to the question "*did the NSEP feel like you were playing netball? If yes, why? If no, why?*" confirmed that 100% of the girls felt as though they would normally do during a netball match, although there was no ball or opponent.

5.4 Discussion

The present study was the first to examine free-living 24-hour energy intake, macronutrient intake, 24-hour energy expenditure, 24-hour energy balance, appetite (hunger, prospective food consumption, fullness) and mood, in trained adolescent netball players including a single bout of netball specific based exercise over a 5-day period. The two major findings of this study were that energy intake following the NSEP over day 3 and day 4 (48-hours) in the NSEP treatment week was elevated when compared to the corresponding days in the SED treatment week and that the participants felt significantly hungrier immediately following the NSEP, compared to immediately before.

5.4.1 Protocol Considerations

Conducting the study period over two 5-day treatment weeks was felt to be the most appropriate time frame for several reasons. A lack of energy intake compensation for a single bout of NSEP within 1 hour of exercise cessation has previously been demonstrated in habitually active girls (12–14 years old) (Rumbold & Dodd, 2007). It was felt that energy intake needed to be monitored over a longer period of time, as energy intake compensation for exercise-induced energy expenditure in lean individuals must occur at some point to prevent continual weight loss (Dodd, 2007). Dodd, Welsman and Armstrong (2008) were the first to monitor energy intake and appetite following cycling exercise in young children, over 5-days (Dodd, Welsman & Armstrong, 2008). Since the study by Dodd, Welsman and Armstrong (2008) was laboratory based, this provided a foundation for further work. There is no information in the literature regarding free-living energy intake, appetite and mood following sport-specific exercise in habitually active adolescent girls over 5-days and subsequently this study was the first to investigate these parameters in this population group. Cooperation from the school and especially the PE staff enabled the girls to participate in the 5-day treatment weeks over two occasions. Since the girls were all part of the school netball squads, a period longer than 5-days would have impacted greatly on availability for school netball matches.

The inclusion of two maintenance days (days 1 and 2) was necessary for several reasons. Initially this duration has been used successfully in adult studies which have monitored self-report energy intake (Stubbs *et al*., 2002a,b; 2004). Importantly, such measures also ensured that any alterations in energy expenditure, energy intake, hunger, prospective food consumption, fullness and mood were a result of the NSEP intervention on day 3 of the NSEP treatment week. Standardization of 24-hour energy expenditure was successfully achieved, since on maintenance days 1 and 2 there were no differences between and within treatment weeks. This was facilitated by liaising with the school staff and parents to ensure the girls remained sedentary and were excused from PE and other ‘active’ lessons. 24-hour energy intake and 24-hour macronutrient intake before each treatment week was also successfully standardised, since on maintenance days 1 and 2 there were no differences between and within treatment weeks. Subsequently, 24-hour energy balance did not differ between or within treatment weeks, on maintenance days 1 and 2. Standardization of hunger, prospective food consumption and fullness was also successful for maintenance days 1 and 2, as there were no differences in ratings between and within treatment weeks. Such energy expenditure, energy intake, macronutrient intake and appetite standardisation ensured that all participants began each week at the same relative nominal point to that of the previous week (Stubbs *et al.*, 2004), allowing any alterations in energy intake behaviour to be accounted for by the exercise-induced energy expenditure imposed on day 3 of the NSEP treatment week. Previous research demonstrates that adult habitual exercisers are able to regulate their compensation in terms of food intake more accurately in response to previous dietary energy intake (King *et al*., 1999; Long, Hart & Morgan, 2002). Although this has not been investigated in young habitual exercisers, the inclusion of maintenance days 1 and 2 to standardize energy intake and macronutrient intake excluded the possibility of this occurring. Such dietary standardization is believed to facilitate the reliability of the VAS ratings (Flint *et al*., 2000) and also provided an opportunity for the girls to become familiar with responding to the appetite and mood related questions.

In terms of the daily mood parameters for maintenance days 1 and 2, significant differences were identified for alertness between the NSEP and SED treatment weeks and for activeness within the NSEP treatment week. More specifically the girls felt significantly more alert on day 1 of the SED treatment week compared to the corresponding day in the NSEP treatment week. Within the NSEP treatment week the girls felt significantly more active on day 2 compared to day 1. Despite this there were no differences for 24-hour energy expenditure, 24-hour energy intake, 24-hour energy balance and daily hunger, prospective food consumption and fullness ratings for maintenance days 1 and 2, thus suggesting that the girls may have felt more alert and more active but that this was not reflected by a compensatory increase or down-regulation in energy expenditure or even an alteration in energy intake values on the corresponding days. Since the majority of parameters were consistent between weeks, it is unlikely that mood state overall was influential on the two weeks' activities.

5.4.2 Energy Expenditure

As intended, energy expenditure was only elevated on day 3 of the NSEP treatment week when compared to all other days within that week and the corresponding day 3 in the SED treatment week, 12.74±1.32 versus 11.07±1.22 $MJ \cdot d^{-1}$, respectively. The large standard deviation values in the energy expenditure data can be accounted for in part by between subject variations, using the FLEX heart rate technique (Livingstone, 1997). For energy expenditure on days 4 and 5, there were no significant differences within or between treatment weeks. Consequently, any adjustments in energy intake in the NSEP treatment week are likely to have been due to energy requirements as a result of an elevated energy expenditure on the NSEP intervention day.

The predicted energy expenditure, using the FLEX heart rate technique, induced by the 47 minute NSEP was 1.44±0.18 MJ compared to 0.33±0.04 MJ in the SED intervention. Due to the self-paced, intermittent nature of the NSEP energy expenditure was expected to vary between participants, which indeed it did, ranging from 1.19 to 1.81 MJ. Additionally, due to the intermittent nature of the NSEP, excess post-exercise oxygen consumption (Dodd, Welsman & Armstrong, 2008) is also likely to vary between individuals thus increasing the variation in energy expenditure quantification throughout the exercise bout demonstrated by the range of energy expenditure values.

Interestingly, the elevated energy expenditure on the NSEP exercise day did not have an overall effect on 72-hour energy expenditure when the treatment weeks were compared. This contradicts previous paediatric findings (Dodd, Welsman & Armstrong, 2008), where the cited authors identified an elevated energy expenditure over 5-days during an exercise week when compared to a sedentary week. A pertinent difference between the present study and the study of Dodd, Welsman and Armstrong (2008) is that the lean girls involved in the later study participated in two consecutive bouts of cycling exercise over two consecutive days, inducing an energy expenditure of 1.35 MJ and 1.60 MJ respectively. Importantly, in the present study the girls only participated in one single bout of NSEP, however the range of energy expenditure values induced by the NSEP (1.19 MJ to 1.81 MJ) encompassed those values imposed by Dodd, Welsman and Armstrong (2008). Thus if two consecutive bouts of NSEP were imposed a similar overall effect on

72-hour energy expenditure to that demonstrated by Dodd, Welsman and Armstrong (2008) may have been identified. In addition, Dodd, Welsman and Armstrong (2008) did not quantify 24-hour energy expenditure, however the present study did, therefore it may be the case that elevations in energy expenditure induced by a single bout of exercise may not manifest itself over a 72-hour period. However as previously discussed, the aim of the present study was to elevate 24-hour energy expenditure on day 3 of the NSEP treatment week, in relation to day 4 and 5 of that week, which was successfully achieved.

The present study was the first study to quantify 24-hour free-living energy expenditure in a paediatric population using a combined FLEX heart rate and resting metabolic rate assessment technique. It is well documented that using the FLEX heart rate technique to estimate energy expenditure has advantages and weaknesses (Livingstone, Robson & Totton, 2000). In terms of the requirements of the present study, energy expenditure for both sedentary and exercise activities needed to be quantified over a short-term period of 5-days. The FLEX heart rate technique when used over a minimum of 3-days, is believed to be the most accurate method to monitor energy expenditure in young people (Livingstone *et al*., 1992a; Maffeis *et al*., 1995). During sedentary pursuits the relationship between heart rate and oxygen uptake is weak (Livingstone, Robson & Totton, 2000), allowing heart rate to be influenced by variables such as posture, emotional state and the environment (Murgatroyd, Shetty & Prentice, 1993; Keytel *et al*., 2005). Therefore, to provide a representative value for energy expenditure during sedentary activities each individual was assigned a FLEX heart rate threshold to account for this, which differentiated between sedentary and activity heart rate values. During exercise however, the relationship between heart rate and oxygen uptake is linear (Christensen *et al*., 1983), providing a representative value for energy expended during an exercise bout. In addition energy expenditure during the NSEP was estimated as precisely as possible since the same movement patterns were used to establish the FLEX heart rate regression line (Ceesay *et al*., 1989). In addition, the FLEX heart rate technique recognises that there is a tenuous relationship between heart rate and $\dot{V}O_2$ at low intensities of energy expenditure, allowing factors such as posture to increase heart rate without an associated increase in $\dot{V}O_2$ and therefore energy expenditure (Livingstone, Robson & Totton, 2000). A solution to such inaccuracies in energy expenditure estimation has shown potential in the form of simultaneous heart rate and motion monitoring using uniaxial accelerometers (Brage *et al*., 2004). In turn, this may improve the estimation of energy expenditure compared to using heart rate data alone (Luke *et al*., 1997; Brage *et al*., 2004).

By extending the FLEX heart rate assessment into a $\dot{V}O_{2\ peak}$ test, exercise intensity throughout the NSEP was then established for each participant. It was not the aim of this study to impose a specific exercise intensity at which the girls were required to exercise at, but rather to quantify what intensity the NSEP induced, taking into account it was designed as a self-paced intermittent replica netball protocol for the respective age group. The mean exercise intensity induced by the NSEP was 64±5% $\dot{V}O_{2\ peak}$ and in terms of percentage of maximum heart rate this equated to 83±6%. The only available published literature concerning netball game intensities is based on national and elite level adult netball players (Woolford & Angove, 1991; 1992), with no comparable published data concerning paediatric groups. From the available literature however it seems that there is no information regarding netball match exercise intensity in terms of percentage $\dot{V}O_{2\ max}$. Monitoring of heart rate during national and international adult netball matches has provided information regarding game intensities in the form of percentage maximum heart rate (Woolford & Angove, 1991). The three elite netball players in the cited study spent the majority of time playing at exercise intensities of between 85%-95% maximum heart rate. However, Woolford and Angove (1991) demonstrated that when player position in one game is inconsistent, this influences the percentage of time spent at different heart rate intensities. The nature of the opposition, when player position is consistent also affects the percentage of time spent at different heart rate intensities, concluding that the exercise intensity of netball match play varies considerably within games due to player position and between games due to the opposition (Woolford & Angove, 1991). The NSEP successfully achieved such variation in terms of exercise intensity defined using percentage of maximum heart rate, since throughout the NSEP the girls were working at 83±6%. Although this did not reflect the typical game intensity identified by Woolford and Angove (1991;1992) of between 85%-95% maximum heart rate, the age of the participants (adolescents versus adults) and the level of competition which was intending to be replicated, community based club level in the present study versus elite level competition, must be considered. Ratings of perceived exertion values during the NSEP demonstrated that the girls felt that the exercise was *starting to get hard* during the second quarter of the NSEP and for the third and fourth quarter they felt it was *getting quite hard*, thus it seems the girls perceived that they were working at a suitable exercise intensity. In support of this, findings from the retrospective questionnaire distributed, confirmed that whilst completing the NSEP all the girls felt as though they would normally do during a netball match, with the only difference being there was no ball or opponent. However, it must be acknowledged that a limitation of the questionnaire was its subjective nature and that neither reliability nor validity of the questionnaire was explored in the present study, as it was only included for evaluative purposes. In addition, heart rate was sampled over 60 second epochs during the NSEP intervention in order for 24-hour energy expenditure quantification to be consistent over the five study days. It is likely high intensity work periods equating to 85% maximum heart rate and above were not identified in the present study since such work periods only last approximately 5 seconds (Allison, 1978). By averaging heart rate over 60 second epochs, such detail may have been lost, however the protocol was novel and successfully served as an exercise bout which mimicked netball competition for adolescent players. It was also necessary to average heart rate over 60 second epochs as heart rate data was required to be collect over five consecutive days and if smaller epoch intervals had have been used there would not have been enough memory to store the data over the 5-day duration.

5.4.3 Energy Intake and 24-hour Energy Balance

The major finding of this study was an elevation in energy intake following the NSEP over day 3 and day 4 (48-hours) in the NSEP treatment week when compared to the SED treatment week. With regards to all the other study days (days 3, 4 and 5) no differences in 24-hour energy intake were found, either between or within treatment weeks. This finding has not been previously identified in paediatric groups, since energy intake following a single exercise bout has only been monitored over 60 minutes (Rumbold & Dodd, 2007) in 12-14 year old girls and one day (Moore *et al*., 2004) in 9-10 year old girls. When the energy intake monitoring period has been extended to 5-days (Dodd, Welsman & Armstrong, 2008), as was the case in the present study, 10-11 year old lean and overweight girls did not demonstrate a compensatory increase in energy intake following two consecutive bouts of high intensity cycling exercise. Unlike the present study, dietary restraint was not assessed in the cited studies therefore it is unknown whether the girls were restricting their food intake intentionally. In addition, the cited studies all directly monitored energy intake by providing meals of a set macronutrient composition and thus controlling when the girls ate. Therefore the major difference between the present study and those by Moore et al. (2004) and Dodd, Welsman and Armstrong (2008) was that free-living energy intake responses to exercise were examined for the first time in adolescent girls. The girls were able to control the types of food they consumed and the frequency of eating occasions (Moore *et al*., 2004). In addition, all the girls were classified as unrestrained eaters. Therefore, taken together, these reasons may be an explanation for the differences in findings between the present study and those of Moore et al. (2004) and

Dodd, Welsman and Armstrong (2008). The only comparable data is that derived from the adult literature.

Free-living energy intake has been monitored in six lean women all unrestrained eaters whilst completing seven consecutive days of high intensity cycling (3 x 40-minutes / 3.4 $MJ \cdot day^{-1}$) (Stubbs *et al.*, 2002b). The cited authors concluded that there was a partial energy intake compensation (~30%) when diet was unrestricted for 7 days. Unlike the present study the level of energy expenditure was high (3.4 $MJ \cdot d^{-1}$) compared to a lower value of 1.44±0.18 MJ for the NSEP intervention. However, the energy cost of exercise when comparing values between adults and adolescents is more appropriately interpreted relative to body mass, thus equating to 20 $kJ \cdot kg^{-1}$in the present study and 42.8 $kJ \cdot kg^{-1}$ in the work of Stubbs et al. (2002b). Even when the energy cost of exercise is examined relative to body mass energy expenditure was considerably lower in the present study compared to that of Stubbs et al. (2002b). The similarity of the studies in terms of the monitoring of free-living energy intake may be the influential factor and may facilitate the identification of a compensatory effect. Durrant, Royston and Wloch (1982) identified that exercise increases the frequency of eating and drinking episodes and thus by monitoring free-living energy intake over several days following an exercise bout, individuals are unrestricted and can consume familiar foods at frequencies and quantities with which they desire. Additionally, in the absence of adult influences the girls may have increased their energy intake by selecting foods and portion sizes which would partially restore energy balance after the exercise bout.

Alongside monitoring free-living energy intake, the group selection criteria in the present study must also be considered. In one of the first appetite and exercise studies, famously cited, Edholm et al. (1955) conducted a cross-sectional study which monitored habitual physical activity and free-living food intake patterns of lean trained male cadets over 14 days. Edholm et al. (1955) identified that energy expenditure on one day was matched with energy intake 2 days later, supporting the notion in the present study that a 48-hour period maybe required in order to identify an elevation in energy intake. This potentially explains why energy intake was only elevated over 48 hours following the exercise bout and not over the 72 hour period which included the exercise bout. Thus the 48-hour energy intake compensation witnessed in the present study may have been a genuine psycho-physiological response in an attempt to maintain energy balance in response to an elevated energy expenditure.

For 24-hour energy balance over days 3, 4 and 5 there were no significant differences between treatment weeks or within treatment weeks, nor were there any significant differences between treatment weeks for 48-hour and 72-hour energy balance. Interestingly, the girls were all in a continuous state of negative energy balance during both the NSEP and SED treatment weeks. On day 3 of the NSEP treatment the girls were more negative compared to the corresponding day in the SED treatment week with the explanation being an elevated energy expenditure as a result of the NSEP intervention. On days 4 and 5 of the NSEP treatment week however all participants were less negative (although not statistically significant) compared to the corresponding days in the SED treatment week. These results along with the energy intake compensation over 48-hours in the NSEP treatment week, demonstrate that lean trained adolescent girls are better able to regulate their energy intake following a period of elevated energy expenditure, in comparison to a sedentary period. Indeed, adult habitual exercisers have demonstrated an ability to regulate their energy intake compensation in response to previous dietary energy intake following exercise (King *et al*., 1999; Long, Hart & Morgan, 2002). Consequently, such sensitivity with regards to energy regulation may also be the case when energy expenditure is manipulated in lean habitually active adolescent girls.

5.4.4 Macronutrient Intake

For protein, carbohydrate and fat, there was no difference in the percentage contribution to daily free-living energy intake for days 3, 4 and 5 following the NSEP and SED interventions. This indicates that the prescribed NSEP had no influence on specific macronutrient selection, since values were consistent between and within weeks. There is no comparable data in terms of macronutrient selection following exercise in paediatric populations, since two studies to date (Moore *et al*., 2004; Rumbold & Dodd, 2007) have provided foods of a set macronutrient composition and the other paediatric study (Dodd, Welsman & Armstrong, 2008) provided set meals in a laboratory setting. These studies have provided a baseline for further work, with the present study being the first to examine free-living macronutrient food choice following netball-specific exercise in a group of habitual adolescent netball players.

The adult literature however demonstrates a preference for carbohydrates 1 to 2 hours following athletic type activities when compared to a sedentary condition (Verger, Lanteaume & Louis-Sylvestre, 1992). Furthermore, protein intake has also been found to increase, after 30 minutes of athletic type exercises (Verger, Lanteaume & Louis-Sylvestre, 1994), demonstrating an inconsistency between the findings of Verger, Lanteaume and Louis-Sylvestre (1994) to the findings of the present study. Unlike the present study, the study by Verger, Lanteaume and Louis-Sylvestre (1992) limited food choice, imposed unrealistic eating situations and employed an independent group design, thus compromising the use of the results. In the present study a repeated measures design was employed and the girls consumed food in a free-living environment, which is a more realistic approach (Hill, Rogers & Blundell, 1995). Consequently, the onset of eating could not be controlled and therefore examination of macronutrient intake immediately after the NSEP and SED interventions was logistically not possible. It was demonstrated in chapter four of this thesis that the girls in the present study could report their free-living energy intake accurately, which emphasizes the robustness of these findings. Since there were no significant differences in macronutrient selection following the NSEP and SED interventions, the increase in energy intake over the 48-hour period in the NSEP treatment week is a result of the girls consuming more food in terms of absolute amount as opposed to selecting certain energy dense food items.

Although carbohydrate intake as a percentage of daily energy intake did not differ significantly following the NSEP and SED interventions, it tended to be higher compared to that of protein and fat over all five study days, in each of the treatment weeks. The macronutrient results of the present study can be compared to dietary reference values for adults (19-64 years old) proposed by the Department of Health (1991) for protein, carbohydrate and fat as a percentage of daily energy intake, 15%, 50% and 35% respectively. This comparison indicates that over the study days in the NSEP and SED treatment weeks, the girls were consuming slightly higher amounts of protein, considerably higher amounts of carbohydrate and lower amounts of fat. Saris (1989) observed a similar increase in carbohydrate intake at the expense of fat in Tour de France cyclists over 21 days. Maughan (1989) also identified an increase in carbohydrate intake over 7 days in male runners. The two cited studies suggest that habitual exercisers are likely to alter the composition of their diet over the longer-term to suit their regular energy expenditure levels. All the girls involved with the study were habitual netball players, completing netball training on a regular basis prior to the study protocol. Netball is described as an interval sport, involving short sprints followed by recovery periods (Allison, 1978). Consequently, the girls will require energy primarily from carbohydrate rich sources in comparison to fat, to continually replenish liver and muscle glycogen stores, enabling subsequent bouts of moderate to high intensity exercise to be completed (McArdle, Katch & Katch, 1991). Indeed, the American Dietetic Association (A.D.A, 1996) proposes that adolescent athletes require 55% to 60% of total energy from carbohydrate in their diet to meet the nutritional demands of physical activity and health. These values therefore support the macronutrient intake of the adolescent girls involved in the present study.

5.4.5 Subjective Appetite

With regards to the study days 3, 4 and 5, there were no significant differences in 24-hour hunger, prospective food consumption or fullness ratings, either between or within treatment weeks. There was also no difference between the NSEP and SED interventions in mean hunger, prospective food consumption and fullness immediately following the NSEP and SED interventions. This has previously been identified in 12-14 year old habitually active girls after completing a single bout of NSEP (Rumbold & Dodd, 2007). The results of the present study also indicated, however, that the girls felt significantly hungrier following the NSEP, compared to immediately before, however differences were not identified for prospective food consumption and fullness. Interestingly, this elevation in hunger following the NSEP bout was not accompanied by an increase in energy intake on day 3 of the NSEP intervention.

Previous findings in the paediatric literature have demonstrated a 'desire to eat less' immediately following a high intensity cycling bout at 75% $\dot{V}O_{2\ peak}$ compared to cycling at 50% $\dot{V}O_{2\ peak}$ and a sedentary condition in a group of 9-10 year old lean girls (Moore *et al.*, 2004). Interestingly this finding has not been replicated since in lean young children rather an increase in hunger immediately following exercise was found in six overweight girls aged 10-11 year old (Dodd, Welsman & Armstrong, 2008). Following cycling at 75% $\dot{V}O_{2\ peak}$ the six overweight girls in the Dodd, Welsman and Armstrong (2008) study felt significantly more hungry and less full immediately following the exercise bout compared to immediately before. Similar to the present study, the changes in the appetite ratings hunger and fullness were not accompanied by an elevation in energy intake following the exercise bouts.

Similar to adults, there does not seem to be a prolonged affect of exercise on subjective appetite ratings in paediatric groups, however in contrast a brief suppression in appetite has not yet been identified in young people but rather an increase in hunger. Further investigation would confirm whether the elevation in hunger finding in the present study is a reflection of the intermittent sport-specific nature of the NSEP completed by the lean habitually active adolescent girls (13-15 years old). Interestingly, the exercise intensity induced by the NSEP was not classified as being of a high intensity (64±5% $\dot{V}O_{2\ peak}$). Indeed in adults, exercising at a high intensity (75% $\dot{V}O_{2\ max}$) seems to be a pertinent factor in eliciting a response in terms of a suppression in hunger following exercise (Thompson, Wolfe & Eikelboom, 1988; King, Burley & Blundell, 1994). Interestingly, however lean young girls 9-10 years old (Moore *et al.*, 2004) and 10-11 years old (Dodd, Welsman & Armstrong, 2008), cycling at a high intensity (75% $\dot{V}O_{2\ peak}$) did not demonstrate a similar change in the subjective appetite parameters. This therefore suggests that the type of exercise and not necessarily exercise intensity maybe an important factor which influences subjective appetite parameters in lean children and adolescents. It may also be the case that like overweight girls, lean trained girls may be less sensitive to the physiological inhibition of appetite following exercise (Dodd, 2007). Alternatively, trained girls may be more sensitive to physiological appetite sensations, which manifest themselves in the subjective appetite parameters, such as feelings of hunger. A possible explanation for this is age (13-15 years old) and consequently the girls may have used their past experiences (Stubbs, Ferres & Horgan, 2000) when responding to the VAS questions. In addition, since the girls were all habitual exercisers this may have contributed to this notion. Additionally, the subjective nature of the visual analogue scales in conjunction with the girls being habitual exercisers may have resulted in them thinking that they should feel more hungry after exercise.

Consequently, due to the ambiguity in the paediatric studies to date with regards to subjective appetite and in particular hunger following exercise, there is a need to objectively assess appetite. Potentially the examination of key appetite hormones such as acylated ghrelin would provide this vital information and provide some clarity into the appetite responses to exercise in young people.

5.4.6 Mood

With regards to the study days 3, 4 and 5, there were no significant differences in 24-hour alertness, activeness, tiredness, happiness, boredom, relaxation and overall mood ratings, either between or within treatment weeks.

The main finding however was that the girls felt significantly more alert immediately after the NSEP intervention compared to immediately after the SED intervention. The consensus from the paediatric literature is that physical activity has short-term benefits on improving concentration (Taras, 2005). Caterino and Polak (1999) divided 54 children (6-9 years old) into two groups, one of which completed 15 minutes of physical activity (stretching and aerobic walking) and a second group who did not. A test of concentration was then administered to all children. Those who were 9 years old demonstrated better performances on the concentration test if they had been in the physical activity group, when compared to their sedentary counterparts. Caterino and Polak (1999) concluded that the improvement in concentration takes place immediately after the exercise period. This was demonstrated in the present study since the girls felt significantly more alert immediately following the NSEP intervention compared to the SED intervention, however when the 24-hour alertness ratings were compared for the overall intervention day (day 3), there were no significant differences between treatment weeks. Therefore it seems an improvement in alertness following exercise is short-lived and in this case a result of the exercise bout and is not influenced by energy intake since energy intake prior to each intervention period was standardised and 24-hour energy intake on day 3 in each treatment week was not significantly different. These findings warrant further work in this area, however unfortunately this is outside the scope of the present series of studies.

5.5 Conclusion

In conclusion, the present study was the first study to impose a single bout of representative intermittent netball exercise on day 3 of a 5-day intervention period (including a 2-day maintenance period and a 3-day follow up period) whereby free-living energy intake, energy expenditure, energy balance, subjective appetite and mood were monitored in a group of adolescent netball players (13-15 years old). The major finding of this study was that the adolescent netball players increased their energy intake over a 2-day (48-hour) period following a bout of netball based exercise when compared to a SED intervention. They also reported that they felt significantly hungrier immediately following the exercise bout. This finding has not previously been identified in lean paediatric or adolescent groups, however it is likely in previous studies that the participants were untrained. Therefore, it would be appropriate to directly compare energy intake and appetite following exercise in trained and untrained adolescent girls. Examination of key appetite hormones such as acylated ghrelin, in conjunction with self-reported hunger, fullness and desire to eat ratings may provide a clearer picture and explanation of appetite responses to exercise in adolescent girls, both trained and untrained.

CHAPTER 6

Comparison of Energy Intake, Subjective Appetite, Acylated Ghrelin, Glucose and Insulin Responses to a Single Bout of Netball-Based Exercise, between Trained Adolescent Netball Players (13-15 years old) and their Untrained Peers, over 7-days

6.1 Introduction

In chapter five of the present work, energy intake and subjective appetite were monitored over a period of 5-days (two maintenance days, followed by a 3-day follow up period, which included a bout of intermittent netball exercise). The major finding of this study was that the adolescent netball players increased their energy intake over a 2-day (48-hour) period when compared to a SED intervention. They also reported that they felt significantly hungrier immediately following the exercise bout. It is clear that such work should now be substantiated using more sensitive measures of energy expenditure quantification, such as a combined heart arte and physical activity method and also a more objective measure of appetite, such as acylated ghrelin.

Acylated ghrelin is a gastrointestinal hormone (28-amino acid peptide hormone) released primarily from cells in the stomach and is the only known orexigenic hormone (appetite stimulant) (Cummings, 2006), stimulating neuropeptide Y and agouti-related peptide and therefore feeding (Wren *et al*., 2001). Consequently, ghrelin is associated with feelings of hunger (Blundell, Goodson & Halford, 2001), with plasma ghrelin levels surging before meals (Cummings *et al*., 2001: Cummings *et al*., 2004; Cummings, 2006) and being suppressed following meals (Cummings & Overduin, 2007). In support of this, ghrelin administration in humans has been demonstrated to stimulate appetite and food intake (Wren *et al*., 2001; Druce *et al*., 2005; Wren *et al*., 2006). Chapter five of the present work identified an elevation in subjective hunger immediately following netball-based exercise in 13-15 year old adolescent girls. This study proposes to be the first to explore plasma ghrelin concentrations following exercise in trained and untrained adolescent girls. All the available research concerning ghrelin responses to exercise has so far only been conducted in adult males and females.

Acylated ghrelin is the most active form of ghrelin, although the majority of circulating ghrelin (80-90%) is non-acylated (Hosoda *et al*., 2004; Ghigo *et al*., 2005). Non-acylated ghrelin is unable to bind and activate the growth hormone-secretagoue receptor and is therefore devoid of any affects on the endocrine axis (Ghigo *et al*., 2005), whereas acylated ghrelin has the ability to bind and therefore cross the blood-brain barrier

(Kojima *et al*., 1999; Murphy & Bloom, 2006). In comparison to acylated ghrelin, non-acylated ghrelin does not possess the pituitary or pancreatic endocrine associated activities and consequently is not considered to be important for appetite regulation (Broglio *et al*., 2003). The majority of adult studies, with the exception of one (Broom *et al*., 2007) have explored total ghrelin concentrations following exercise and not acylated ghrelin concentration following exercise. Thus acylated, as opposed to total ghrelin should be assessed in further work with regards to exercise, especially where a new special population i.e. adolescent girls is concerned.

It is also necessary to extend the study period over a period of 7-days (two maintenance days, followed by a 5-day follow up period) since partial energy intake compensation (~30%) has been identified in previous 7-day studies involving adult females (Stubbs *et al*., 2002b). Indeed, in chapter five of the present work an elevation in energy intake was identified over 2-days following an exercise bout in adolescent girls. However this was not identified over the 3-day follow up period, therefore it is necessary to extend this study period beyond 3-days. In addition, only two paediatric studies (Rumbold & Dodd, 2007; chapter five of the present thesis) have explored energy intake and appetite following exercise in trained adolescent girls, however as yet none have compared trained and untrained adolescent girls directly.

It has been suggested that if individuals are untrained a disruption in energy homeostasis is likely to occur (Mayer, Roy & Mitra, 1956; Mugatroyd, 1999; Shephard *et al*., 2001; Stubbs *et al*., 2004). Support for the role of exercise in appetite regulation has been provided by King et al. (1999). Following 50 minutes of running at 70% $\dot{V}O_{2\ max}$, the cited authors provided 16 male habitual exercisers (21.3 ± 12.4 years) with a drink of water, a low-energy drink or a high-energy drink, using a repeated measures design. The participants significantly increased their energy intake following the low-energy drink, compared to the water and high-energy drink trials. This demonstrates an enhanced ability of habitually active groups of individuals to decrease their energy intake (by approximately the energy content of the high-energy drink) at a subsequent test meal when a high-energy drink is consumed after exercise. Long, Hart and Morgan (2002) conducted a similar study however they explored the differences between habitually active and habitually non-active individuals, without imposing an exercise bout. High and low energy liquid preloads were provided to the groups, followed 60 minutes later with an *ad libitum* buffet meal. Individuals who were habitually active significantly reduced their energy intake following the high-energy preload compared with the low. This was not however the case for habitual non-exercisers. Taken together, these results suggest that regardless of whether an exercise bout is imposed, habitually active individuals are more accurate in regulating their appetite and energy intake through compensation for previous dietary energy pre-loads of varying calorific content. Indeed, when habitually non-active males were enrolled on a 6-week exercise regime the above findings were reiterated (Martins, Truby & Morgan, 2007), thus demonstrating the sensitising effect of exercise on appetite regulation certainly in terms of responses to previous dietary intake (King *et al*., 1999; Long, Hart & Morgan, 2002; Martins, Truby & Morgan, 2007). It has been postulated that there are differences in hormonal responses to exercise, between habitually active and habitually non-active cyclists (Bloom *et al*., 1976), which may be a contributory factor in energy intake responses to exercise-induced energy expenditure. Jürimäe, Jürimäe and Purge (2007) suggest that the release of ghrelin relating to exercise may differ due the habitual activeness of individuals. Indeed, in studies that have explored plasma ghrelin levels in relation to high-intensity aerobic exercise in habitually non-active individuals, there has been no influence on ghrelin concentrations (Kallio, Pesonen & Karvonen, 2001; Dall *et al*., 2002; Schmidt, Maier & Schaller, 2004). Interestingly, even when individuals have been classified as being habitually active, high-intensity exercise has had no influence on ghrelin concentrations (Kraemer *et al*., 2004; Burns *et al*., 2007). However, in studies that have identified an increase (Christ *et al*., 2006; Jürimäe *et al*., 2007b; Jürimäe, Jürimäe & Purge, 2007) or a decrease (Vestergaard *et al*., 2007) in ghrelin levels in relation to exercise, the participants were performing sports or exercises that they were trained in, as opposed to only being classified as habitually active.

Therefore, the overall purpose of the present study, was firstly to quantify 24-hour energy expenditure, by assessing heart rate and physical activity (via uniaxial accelerometry) simultaneously. Secondly, to explore appetite responses to the netball-based exercises objectively, acylated ghrelin concentrations, along with glucose and insulin concentrations need to be explored, in adolescent populations. Finally, an ad libitum test meal, corresponding with the time of onset of eating (identified in chapter five of the present thesis) needs to be provided following the NSEP and SED interventions to explore the relationship between acylated ghrelin concentrations and energy intake.

Consequently, in a group of trained adolescent girls (13-15 years old) and a group of untrained adolescent girls (13-15 years old), the main aims of the present short-term intervention study were as follows. Firstly, a 2-day maintenance period was imposed prior to a NSEP and SED intervention, whereby free-living 24-hour energy intake, macronutrient intake, 24-hour energy expenditure, 24-hour energy balance and subjective appetite (hunger, prospective food consumption and fullness) were monitored in the trained and untrained girls. Secondly, subjective appetite (hunger, prospective food consumption and fullness) and objective measures of appetite (acylated ghrelin, glucose and insulin) were investigated before and after a NSEP intervention and also immediately after a NSEP and SED intervention, in the trained and untrained girls. Thirdly, the relationships between acylated ghrelin and parameters such as glucose, insulin, subjective hunger, prospective food consumption and energy intake at an *ad libitum* test meal, were examined in the trained and untrained girls. Over 5-days following a NSEP and SED intervention, free-living 24-hour energy intake, 24-hour energy expenditure, 24-hour energy balance, daily macronutrient selection (fat, carbohydrate and protein) and daily subjective appetite (hunger, prospective food consumption and fullness) were monitored in the trained and untrained girls. Finally, total 2-day (48-hour) energy intake and 2-day (48-hour) energy balance, total 3-day (72-hour) energy intake and 3-day (72-hour) energy balance, total 4-day (96-hour) energy intake and 4-day (96-hour) energy balance and total 5-day (120-hour) energy intake, 5-day (120-hour) energy expenditure and 5-day (120-hour) energy balance, were monitored in the trained and untrained girls.

Therefore, the overall aim of this study was to establish whether free-living energy intake, subjective appetite and acylated ghrelin concentrations would be influenced by a bout of netball specific exercise in a group of trained adolescent netball players and their untrained peers.

6.2 Methods

6.2.1 Design

Using a between-subject, randomised cross over design, the study examined a 7-day (two maintenance days and a 5-day follow-up period) dietary observation period in relation to a NSEP and a SED intervention.

6.2.2 Participants

The study was approved by the School of Psychology and Sport Sciences Research Ethics Committee at the University of Northumbria. Written informed consent was obtained from both the participants and their parents or guardians prior to data collection. Both parties were provided with information sheets detailing the procedures and requirements of the study. They were informed that the study was based on general diet and exercise but not told that it was concerned with specific energy intake, following an exercise bout.

Ten participants aged 13-15 years old, were recruited from a local secondary school. Inclusion in the study was based on four criteria, outlined in chapter 5, section 5.2.2).

In addition to the above criteria, researchers liaised with the physical education teachers at the respective school and when necessary netball club or county coaches to determine each girl's level of participation in netball-based exercise. For four consecutive school weeks immediately prior to the testing protocol, attendance at extra-curricular netball training sessions within school time and attendance at out of school community (and county if applicable) netball clubs was examined using school registers and netball club registers, respectively. Five participants were then classified as being netball trained, whilst the remaining five participants were classified as being untrained. The participants that were classified as being trained in terms of netball participation were identified as those that participated in netball training or competition at least three times on a weekly basis in and out of school time (similar to the criteria imposed in chapter five of the present thesis). The remaining five participants who were classified as being untrained, were only involved in physical education lessons at school, thus they were not habitually involved in any additional exercise and indeed netball training or competition out of school time. To confirm that five of the participants were indeed habitually more active than their five habitually less-active peers, the girls wore an Actigraph^{TM} GT1M monitor (Actigraph^{TM}, LLC, Pensacola, Florida, USA) for seven consecutive days prior to the start of the intervention in order to obtain a mean daily physical activity count. The data for this is presented in section 6.3.

6.2.3 Study Protocol

For logistical reasons the 10 girls were tested in two groups of five, which corresponded with them being trained and untrained. The girls remained in these groups for all subsequent testing procedures. Therefore, the study protocol was conducted twice. Two preliminary weeks took place before the intervention period. The first preliminary week involved the tracking of physical activity behaviour to ensure that five of the girls were indeed habitually more active than their five habitually less-active peers. The second preliminary week involved a FLEX heart rate and physical activity calibration visit and a resting metabolic rate measurement. The intervention period involved two 7-day treatment weeks (Saturday - Friday) separated by 1 week. The first treatment week (A) commenced at least 7-days after the FLEX heart rate and physical activity calibration had taken place as shown in table 6.1.

Preliminary Week 1	Preliminary Week 2	Week 3	Week 4	Week 5
Seven consecutive days of physical activity monitoring using an Actigraph™ GT1M monitor. Consultation with the designated doctor with regards to the cannulation.	Body composition assessment (BodPod). FLEX heart rate, physical activity calibration and resting metabolic rate measurement. NSEP habituation.	Treatment week A, NSEP or SED intervention.	Wash out week.	Treatment week B, NSEP or SED intervention.

Table 6.1. Study protocol. Study protocol conducted twice for logistical reasons. Netball specific exercise protocol (NSEP), sedentary (SED).

6.2.4 Preliminary Week 1

To confirm that five of the participants were indeed habitually more active than their five habitually less-active peers, all participants wore an Actigraph™ GT1M monitor (Actigraph™, LLC, Pensacola, Florida, USA) for seven consecutive days prior to preliminary week 2 in order to obtain a mean daily physical activity count. The participants wore the accelerometer on an elastic waist strap, which was positioned over the right hip of each participant (Puyau *et al*., 2002; Trost, McIver & Pate, 2005). Physical activity data was stored at 2 second epochs, which is appropriate to track young people's spontaneous and sporadic habitual activity patterns (Fawkner & Armstrong, 2007; Rowlands & Eston, 2007).

6.2.5 Preliminary Week 2: Day 1

Familiarisation

The participants were familiarised with the VAS and food diaries and also the NSEP as per section 5.2.4. In addition, the participants were told how to use and where to wear the Actigraph™ GT1M monitor. The participants and their parents/guardians were also provided with the opportunity to discuss the cannulation procedure (used to obtain the acylated ghrelin blood samples) with the medical doctor prior to the start of the study.

Body Composition Assessment

Body composition was assessed as per section 3.2.3.

Maturity Offset

Maturity offset was assessed as per section 3.2.4.

FLEX Heart Rate Calibration, Physical Activity Calibration and Peak Oxygen Uptake Assessment

The FLEX heart rate calibration and peak oxygen uptake assessment were assessed as per section 5.2.4. A physical activity count value, which corresponded with the FLEX heart rate threshold value was also obtain for each participant. Physical activity counts were recorded alongside heart rate and oxygen uptake, using an Actigraph™ GT1M monitor, which measured acceleration in the vertical plane of movement and is thus described as a uniaxial accelerometer (Rowlands & Eston, 2007). Using an elastic waist strap, the Actigraph™ GT1M monitor was positioned over each participant's hip, as close as possible to the centre of gravity (Puyau *et al*., 2002; Trost, McIver & Pate, 2005).

6.2.6 Preliminary Week 2: Day 2

Resting Metabolic Rate

Resting metabolic rate was assessed as per section 5.2.5.

6.2.7 Organisation of the Treatment Weeks

Weeks 3 and 5 were deemed treatment week A and B, respectively and were organised as shown in figure 6.1. During the two 7-day treatment weeks, half the participants completed the NSEP whilst the other half remained SED at any one time. Days 1 and 2 (Saturday and Sunday) were deemed maintenance days, with day 3 (Monday) involving a single bout of the NSEP or SED activities. Days 4, 5, 6 and 7 (Tuesday, Wednesday, Thursday and Friday) were classified as follow-up days.

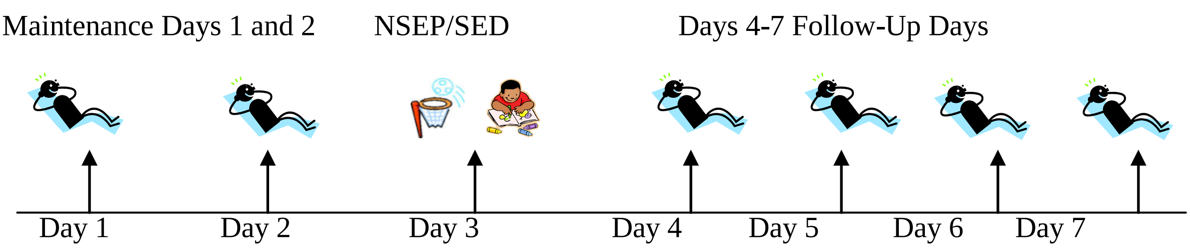


Figure 6.1. Organisation of treatment weeks.

Days 1 and 2 (Maintenance days)

Energy intake, energy expenditure and appetite were standardized as per section 5.2.6. In addition physical activity counts over 60 second epochs using an Actigraph™ GT1M monitor were also measured during the waking hours of these days to ensure that there were no differences in energy expenditure over both maintenance days and between the two treatment weeks.

Day 3 NSEP and SED Interventions

Day 3 was deemed the intervention day whereby NSEP or SED activities were undertaken. Extensive details regarding the structure and organisation of the NSEP are provided in chapter five of the present work. At 08:15 hours, the girls were collected from school and were transported to the university for 09:00 hours. The participants were asked to follow their normal morning routine with regards to breakfast, which was consumed on average at 07:19 hours on day 3 of the NSEP treatment week and at 07:29 hours on day 3 in SED treatment week. At 10:00 half the group completed the NSEP and half were supervised whilst participating in SED activities for the duration of the 47-minute exercise period. Sedentary activities included reading or completing homework tasks. Throughout both interventions, various parameters were measured. These included minute-by-minute heart rate and the collection of physical activity counts over 60 second epochs. Subjective hunger, prospective food consumption and fullness using VAS were assessed immediately before and after the NSEP and SED interventions, and at 10, 20 and 30 minutes during each intervention. Only with regards to the NSEP, the Pictorials Children's Effort Rating Table (Yelling, Lamb & Swaine, 2002) was administered immediately before and after and at 10, 20 and 30 minutes during the protocol, to provide an indication of ratings of perceived exertion, whilst heart rate was recorded simultaneously. The scale ranged from 1 to 10, 1 depicting the exercise as being *very, very easy* and 10 as being *so hard I'm going to stop.* Following the completion of the NSEP and SED interventions at approximately 10:47 hours, the participants rested for 1 hour (sitting reading, watching DVD's or completing school work) and were then offered a test meal at 11:47 hours. The participants had 1 hour to consume the test meal before being returned to school.

When the participants arrived at the laboratory on the Monday (day 3) morning (09:00 hours) of week 3 and week 5 (prior to the NSEP and SED interventions), a cannula was inserted into an antecubital vein, whilst in a semi-supine position, by a qualified medical doctor. This allowed 1 hour for the cannulation of five girls. Each participant had their cannula inserted in a designated area, out of view of the other participants. If a participant had symptoms of feeling nauseous and dizziness, or a blood sample could not be obtained at the required time point, they were withdrawn from the study with regards to the blood analysis. Subsequently, one trained participant and two untrained participants withdrew at this point leaving four trained participants and three untrained participants. Following successful insertion, venous blood samples were collected in precooled 4.9-mL EDTA monovettes, immediately before and immediately after the NSEP and corresponding SED intervention and then at 15 minutes, 30 minutes, 45 minutes and 60 minutes after each intervention. The cannula was kept patent using saline solution after each blood collection. All blood samples were collected whilst the participants were in a semi-supine position. The EDTA monovettes were then spun at 1,681 *g* (4,000 revs·min^{-1}) for 10 minutes in a refrigerated centrifuge at 4^{o}C. The plasma supernatant was then aliquoted into Eppendorf tubes and then stored at -80 ^{o}C for analysis of glucose and insulin.

In separate 4.9-mL monovettes, additional blood samples were drawn at the same time points as described above in order to determine plasma acylated ghrelin concentration. These monovettes contained EDTA and *p*-hydroxymercuribenzoic acid to prevent degradation of the acylated ghrelin by protease. These monovettes were spun at 1,287 *g* (3,500 revs·min^{-1}) for 10 minutes in a refrigerated centrifuge at 4 ^{o}C. The plasma supernatants were then aliquoted into Eppendorf tubes and 100 µl of 1M HCl was then added per mL of plasma. Samples were then be spun at 1,287 *g* (3,500 revs·min^{-1}) for a further 5 minutes. These were then stored at -80^{o}C.

Days 4, 5, 6 and 7 (Intervention day and follow-up days)

Energy intake, energy expenditure and appetite on days 4, 5, 6 and 7 were assessed as per section 5.2.6. In addition physical activity counts collected over 60 second epochs, using an Actigraph^{TM} GT1M monitor were recorded during the waking hours of these days.

6.2.8 Simultaneous Heart Rate and Physical Activity Monitoring

Heart rate was measured as per section 5.2.7. Alongside the monitoring of heart rate, physical activity counts were concurrently recorded at epochs of 60 seconds, using an Actigraph^{TM} GT1M monitor. The participants were required to wear one Actigraph^{TM} GT1M monitor for the waking hours of the seven study days (Saturday – Friday). During sleeping periods the participants were not required to wear the heart rate monitors, heart rate receivers or Actigraph^{TM} GT1M monitor, as it was demonstrated in a pilot study that the participants found wearing the monitors at night, uncomfortable. The Actigraph^{TM} GT1M accelerometer was worn over the right hip of each participant, since this was more practical compared to a lower back positioning. This is especially important in terms of comfort and thus compliance with wearing the accelerometer, as the participants were mainly involved in sedentary activities such as lying supine and sitting, where consequently a lower back positioning of the accelerometer would have been uncomfortable. Data was retrieved using a USB connector cable via the Actilife Data

Analysis Software (Version 3.5.0, Actigraph™, LLC, Pensacola, Florida, USA). An example of accelerometer count data is provided in appendix F.

6.2.9 Estimation of Energy Expenditure

The estimation of energy expenditure using the heart rate data was conducted as per section 5.2.8. For each participant a physical activity count threshold value was also determined, which was calculated in the same manner as the FLEX heart rate threshold value and therefore corresponded with this value. Thus, the heart rate data was interpreted with regards to the physical activity count threshold value. Therefore, when there was an elevation in heart rate without an associated increase in physical activity counts, indicated by a physical activity count which fell below the physical activity count threshold value, energy expenditure was calculated as the equivalent of the mean oxygen consumption of the resting activities (lying supine, sitting and standing) (Ceesay *et al.*, 1989). This scenario indicated that extraneous variables other than physical activity were likely to have caused the elevation in heart rate to occur, which is not necessarily associated with an elevation in oxygen uptake and thus energy expenditure (Livingstone, Robson & Totton, 2000).

6.2.10 Estimation of Energy Intake

Estimation of energy intake was made as per section 5.2.9.

6.2.11 Subjective Appetite

Subjective appetite over the seven study days was assessed as per section 5.2.10. With regards to the intervention periods (NSEP and SED) on day 3, the participants were asked to complete the subjective appetite VAS immediately before and after the NSEP and SED and at 10 minute intervals throughout the duration of both interventions (47 minutes).

6.2.12 Test Meal

The test meal, which the participants were provided with, was given 1 hour after completion of the NSEP and SED interventions at approximately 11:47 hours. The foods and portion sizes provided were based on food and drink items and portions recorded, using self-reported weighed food records by the girls themselves, on the corresponding day (Monday) of the previous week at lunchtime. The foods included white pasta in a tomato and herb sauce with grated mild cheddar cheese. Based on the self-reported weighed food records each food item was provided in excess by half a portion, which equated to 503g cooked pasta, 112g of tomato and herb sauce and 30g of cheese (4.07 MJ). The macronutrient content of the meal provided was 76.5% carbohydrate, 16.5% protein and 7.0% fat. In chapter five of the present thesis, the time of free-living eating onset following the NSEP and SED interventions was 2:13±0.16 and 1:59±0.05 hours, respectively. As far as was logistically possible these time frames were replicated. Consequently, the test meal which the participants were provided with was given 1-hour after completion of the NSEP and SED interventions (11:47 hours). Therefore, the participants were then given until 13:00 hours (in total following the NSEP and SED interventions a period of 2 hour and 13 minutes), to consume the test meal. This time period was consistent across the NSEP and SED interventions.

6.2.13 Exercise Intensity, Ratings of Perceived Exertion and Heart Rate

Exercise intensity was calculated as per section 5.2.12. Mean ± SD values for ratings of perceived exertion using the Pictorial Children's Effort Rating Table (Yelling, Lamb & Swaine, 2002) and the corresponding heart rate ($b \cdot min^{-1}$), were also calculated for immediately before the NSEP intervention, after every 10 minute quarter during the NSEP and immediately after the NSEP intervention.

6.2.14 Blood Biochemistry, Analysis and Disposal

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Breton-neux, France; supplied by Immuno Diagnostic Systems). See appendix G for details of how the acylated ghrelin assay was conducted. Plasma glucose concentrations were determined using enzymatic and colorimetric methods (Biosen, EFK, Diagnostics), whilst plasma insulin concentrations were determined using solid-phase Enzyme Amplified Sensitivity Immunoassay performed on microtiter plates (BioSource, INS-EASIA, Europe S.A). Samples from each participant were analyzed in the same run, to eliminate interassay variation. The within-batch coefficients of variation for the assays were 9.1% and 8.1% for acylated ghrelin, plate one and two respectively and 5.3% for insulin. After the blood samples had been analysed, they were autoclaved then disposed of in clinical waste.

6.2.15 Statistical Analysis

The statistical package SPSS-PC (SPSS Inc., Chicago, IL) was used for all data analyses. Prior to analysis all data was assessed for normality using the Shapiro-Wilks' test. Means ±

SD were calculated for all data. Between groups physical characteristics were compared using a one-way ANOVA.

Days 1 and 2 (Maintenance days)

Mean daily 24-hour energy balance ($MJ \cdot d^{-1}$) was calculated by subtracting 24-hour energy expenditure ($MJ \cdot d^{-1}$) from 24-hour energy intake ($MJ \cdot d^{-1}$) for days 1 and 2. Mean daily 24-hour energy intake ($MJ \cdot d^{-1}$), 24-hour energy expenditure ($MJ \cdot d^{-1}$), 24-hour energy balance ($MJ \cdot d^{-1}$) were analysed for main effects and interactions using a 2 (intervention: NSEP v SED) x 2 (maintenance day) x 2 (trained v untrained) ANOVA (within-within-between design).

For each macronutrient (percentage of daily energy intake), protein, carbohydrate and fat consumed during the two maintenance days a 2 (intervention: NSEP v SED) x 2 (maintenance day) x 2 (trained v untrained) ANOVA (within-within-between design) was used.

For each of the appetite parameters an average of all the time points sampled (immediately upon waking each day, before meal, after meal and finally immediately before bed each night) for each of the maintenance days, in both the NSEP and SED treatment weeks, for trained and untrained participants was calculated, to provide a mean total daily hunger, prospective food consumption and fullness rating. Hunger, prospective food consumption and fullness were then analysed using a 2 (intervention: NSEP v SED) x 2 (maintenance day) x 2 (trained v untrained) ANOVA (within-within-between design).

Day 3 NSEP and SED Interventions

Mean ± SD energy expenditure for the 47 minute NSEP and SED intervention periods was calculated for the trained and untrained girls, and was analysed using a 2 (intervention: NSEP v SED) x 2 (trained v untrained) ANOVA (within-between design). Exercise intensity, ratings of perceivd exertion and heart rate during the NSEP were compared using a between groups one-way ANOVA to address any differences between the trained and untrained participants.

Mean energy intake at the test meal following each intervention, in the trained and untrained participants were calculated. Subsequently, a 2 (intervention: NSEP v SED) x 2 (trained v untrained) ANOVA (within-between design) was conducted.

Mean values for hunger, prospective food consumption and fullness, in the trained and untrained participants were calculated for immediately before each intervention (NSEP and SED), 10 minutes, 20 minutes, 30 minutes during, immediately after the each intervention and at 15 minutes, 30 minutes, 45 minutes and 60 minutes post each intervention on day 3. More specifically, for each appetite parameter a 2 (intervention: NSEP v SED) x 9 (time point) x 2 (trained v untrained) ANOVA (within-within-between design) was conducted.

Mean values for acylated ghrelin, insulin and glucose in the trained and untrained participants were calculated for immediately before each intervention (NSEP and SED), immediately after each intervention and at 15 minutes, 30 minutes, 45 minutes and 60 minutes post each intervention on day 3. Due to the uneven group numbers (trained, n=4) and untrained, n=3), a separate 2 (intervention: NSEP v SED) x 6 (time point) ANOVA (within-within design) was conducted for acylated ghrelin, in the trained participants and a 2 (intervention: NSEP v SED) x 5 (due to a missing time point in the SED intervention) (time point) ANOVA (within-within design) was conducted for acylated ghrelin, in the untrained participants. In addition, for insulin and glucose, separate 2 (intervention: NSEP v SED) x 6 (time point) ANOVA’s (within-with design) were conducted for the trained and untrained girls. Post-hoc Tukey tests could not be conducted for any significant main effects due to the low sample size in each group, therefore the two groups were combined to form one (n=7) for further analysis.

Once again, due to the low sample size in each group (trained, n=4; untrained, n=3), the acylated ghrelin concentrations were analysed for all the participants as one group (n=7) using a 2 (intervention: NSEP v SED) x 5 (due to a missing time point in the SED intervention) (time point) ANOVA (within-within design). This was also the case for insulin and glucose, however as all time points were successfully collected separate 2 (intervention: NSEP v SED) x 6 (time point) ANOVA’s (within-within design) were used.

Pearson’s product moment correlation coefficients were also used to examine relationships between plasma acylated ghrelin concentrations and BMI, body mass, percentage body fat, maturity offset, plasma insulin, plasma glucose, subjective hunger, prospective food consumption, and test meal energy intake, in the trained and untrained girls. Pearson’s product moment correlation coefficients were also used to correlate 45 minute post acylated ghrelin concentrations with test meal energy intake in the two groups, in each intervention, due to the missing 60 minute post acylated ghrelin sample in the SED intervention.

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Mean daily 24-hour energy balance ($MJ \cdot d^{-1}$) was calculated by subtracting 24-hour energy expenditure ($MJ \cdot d^{-1}$) from 24-hour energy intake ($MJ \cdot d^{-1}$) for days 3, 4, 5, 6 and 7. Mean daily 24-hour energy intake ($MJ \cdot d^{-1}$), 24-hour energy expenditure ($MJ \cdot d^{-1}$) and 24-hour energy balance ($MJ \cdot d^{-1}$) were analysed for main effects and interactions using a 5 (week day) x 2 (intervention: NSEP v SED) x 2 (trained v untrained) ANOVA (within-within-between design). Further post-hoc analyses included repeated measures ANOVA and Tukey tests as appropriate.

For day 3 and day 4, 24-hour energy intakes ($MJ \cdot d^{-1}$) were combined to provide a value for 2-day (48-hour) energy intake (MJ). Two day (48-hour) energy balance (MJ) was also calculated for day 3 and day 4. Similarly for day 3, day 4 and day 5, 24-hour energy intakes ($MJ \cdot d^{-1}$) were combined to provide a value for 3-day (72-hour) energy intake (MJ), whilst a 3-day (72-hour) energy balance (MJ) value was also calculated for the corresponding days. In addition, 4-day (96-hour) (day 3, day 4, day 5 and day 6) energy intake (MJ) was also calculated in addition to 4-day (96-hour) energy balance (MJ). Finally, 5-day (120-hour) (day 3, day 4, day 5, day 6 and day 7) energy intake (MJ) was calculated in addition to 5-day (120-hour) energy expenditure (MJ) and 5-day (120-hours) energy balance (MJ). Subsequently, 2-day, 3-day and 4-day energy intake (MJ) and energy balance (MJ) were analysed using a 2 (intervention: NSEP v SED) x 2 (trained v untrained) ANOVA (within-between design), with 5-day energy intake (MJ), energy expenditure (MJ) and energy balance (MJ) being analysed in the same way.

For each macronutrient (percentage of daily energy intake), protein, carbohydrate and fat, for day 3, 4, 5, 6 and 7 in the trained and untrained participants, a 2 (intervention: NSEP v SED) x 5 (week day) x 2 (trained v untrained) ANOVA (within-within-between design) was used to identify any main effects and interactions.

For each of the appetite parameters an average of all the time points sampled during day 3, 4, 5, 6 and 7 (immediately upon waking each day, before meal, after meal and finally immediately before bed each night) in both the NSEP and SED interventions was calculated, to provide a mean daily hunger, prospective food consumption and fullness rating, for the trained and untrained girls. These were then analysed for main effects and interactions using a 5 (week day) x 2 (intervention: NSEP v SED) x 2 (trained v untrained) ANOVA (within-within-between design). Further post-hoc analyses included repeated measures ANOVA, one way independent ANOVA and Tukey tests as appropriate.

For all factorial ANOVA's (between-within design) a Mauchly's sphericity test was conducted and the Greenhouse-Geisser correction was applied if the assumption of sphericity had been violated. When significant differences had been identified, Cohen's d effect size for one-way ANOVA was calculated and interpreted against the effect size categories of ≤ 0.1 = small effect, ~ 0.25 = moderate effect and ≥ 0.4 = large effect (Cohen, 1988; 1992). The significance level was set at $p<0.05$ for all analyses.

6.3 Results

The age and physical characteristics of the trained and untrained participants are provided in table 6.2.

Table 6.2. Age and physical characteristics of the trained (n=5) and untrained (n=5) participants.

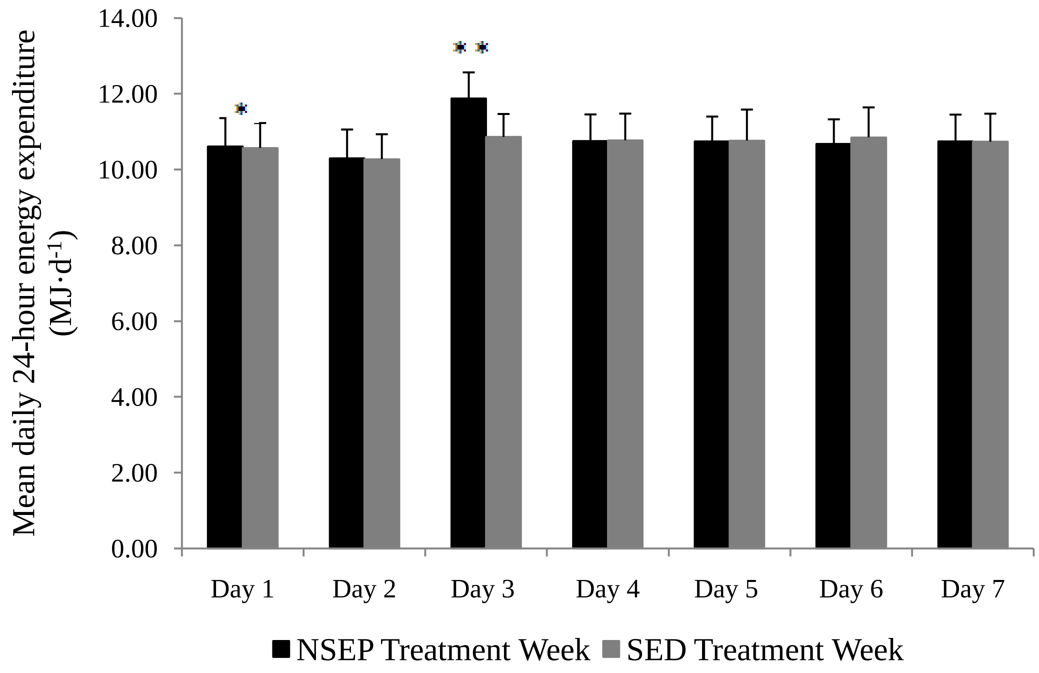
	Trained Participants (n=5)		Untrained Participants (n=5)	
	Mean	**SD**	**Mean**	**SD**
Age (years)	14.1	1.1	15.1	0.8
Stature (m)	1.60	0.10	1.70	0.10
Mass (kg)	53.8	7.4	51.6	6.7
BMI (kg/m^2)	20.0	1.8	18.0	1.3
Body Fat (%)	18.6	1.3	18.1	4.3
Resting metabolic rate (MJ)	6.47	0.49	5.77	0.54
Maturity Offset (years)	1.7	0.9	2.6	0.9
Dietary Restraint	2.0	1.0	1.9	1.0
$\dot{V}O_{2\,peak}$ **($ml\cdot kg^{-1}\cdot min^{-1}$)**	35.6	3.6	36.1	3.5
Mean daily physical activity count ($1\cdot day^{-1}$)	5894.1	829.0*	4920.2	1001.5

***Significantly higher in the trained girls compared to the untrained girls (*p*=0.024).**

6.3.1 Energy Expenditure

Mean ± SD daily 24-hour energy expenditure for each day in each treatment week, for trained participants and untrained participants is provided in figures 6.2a and 6.2b, respectively.

(a)



(b)

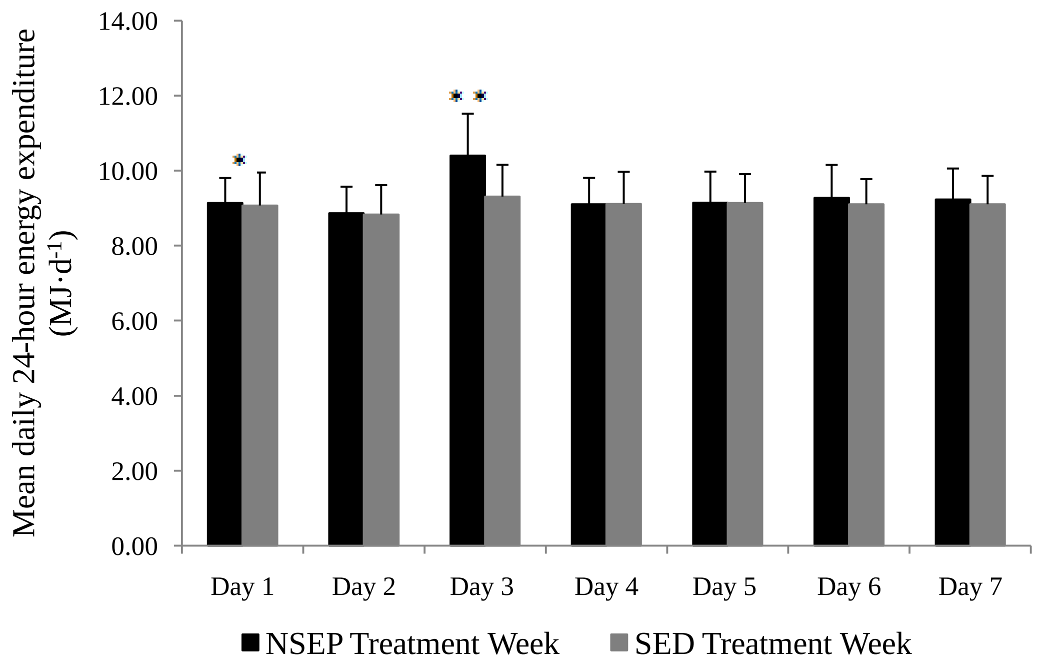


Figure 6.2. Mean ± SD daily 24-hour energy expenditure (MJ) for (a) trained (n=5) and (b) untrained participants (n=5), for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. *Significantly elevated on day 1 compared to day 2 in the NSEP and SED treatment weeks (*p*<0.05). **Significantly elevated between treatment weeks (*p*<0.001) and all other days in the NSEP treatment week (*p*<0.01).

Days 1 and 2 (Maintenance days)

For daily 24-hour energy expenditure for the maintenance days 1 and 2, there were no significant main effects of intervention [$F(1,8)=0.311$, $p=0.592$] or interactions between intervention and training status [$F(1,8)=0.016$, $p=0.903$], week day and training status [$F(1,8)=0.085$, $p=0.778$] or intervention, week day and training status [$F(1,8)=0.004$, $p=0.953$]. There was however a main effect of week day [$F(1,8)=12.428$, $p=0.008$]. Post-hoc Tukey tests identified that energy expenditure was elevated on day 1 ($p<0.05$) compared to day 2 in the NSEP and SED treatment weeks, 9.87±1.02 versus 9.58±1.02 $MJ \cdot d^{-1}$ and 9.82±1.08 versus 9.56±1.02 $MJ \cdot d^{-1}$, respectively.

Day 3 NSEP and SED Interventions

There was a main effect of intervention with regards to the exercise-induced energy expenditure in both the trained and untrained participants. Average exercise-induced energy expenditure was significantly higher for the 47 minute NSEP intervention compared to the 47 minute SED intervention; 1.18±0.20 versus 0.37±0.04 MJ and 1.23±0.26 versus 0.31±0.03 MJ, in the trained and untrained participants respectively [$F(1,8)=142.442$, $p<0.001$, effect size = 5.63].

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

For day 3, 4, 5, 6 and 7 daily 24-hour energy expenditure, significant main effects were found for intervention [$F(1,8)=8.424$, $p=0.020$] and day [$F(4,32)=34.585$, $p<0.001$]. A significant interaction was found for intervention by week day [$F(4,32)=26.293$, $p<0.001$]. Further one way repeated measures ANOVA identified that energy expenditure was elevated on day 3 in the NSEP treatment week when compared to the corresponding day in the SED treatment week, 11.14±1.17 versus 10.09±1.07 $MJ \cdot d^{-1}$, respectively [$F(1,8)=50.829$, $p<0.001$, effect size = 3.36]. Post-hoc Tukey tests also confirmed an elevated energy expenditure for day 3 when compared to day 4 ($p<0.01$), day 5 ($p<0.01$), day 6 ($p<0.01$) and day 7 ($p<0.01$) within the NSEP treatment week, 11.14±1.17 versus 9.93±1.09, 9.95±1.10, 9.98±1.04 and 9.99±1.08 $MJ \cdot d^{-1}$, respectively.

For total 5-day energy expenditure when the NSEP treatment week was compared to the SED treatment week, there was a significant main effect found for intervention but not training status. Total 5-day energy expenditure was elevated in the NSEP treatment week compared to the SED, 50.98±5.38 versus 49.88±5.56 MJ, respectively [$F(1,8)=8.424$, $p=0.020$, effect size = 1.37].

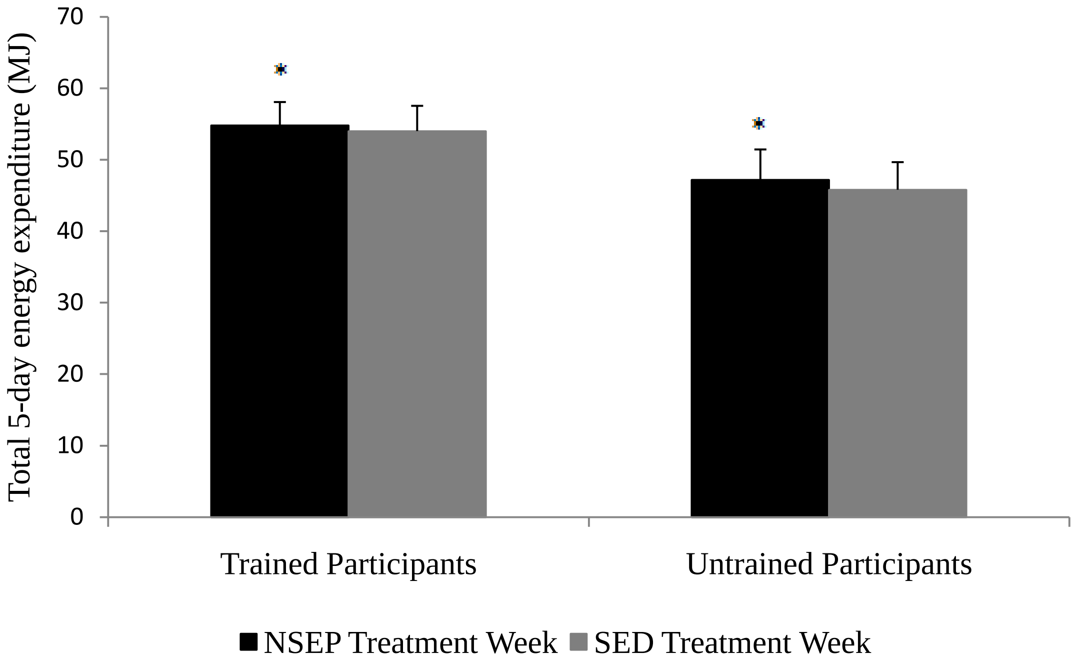
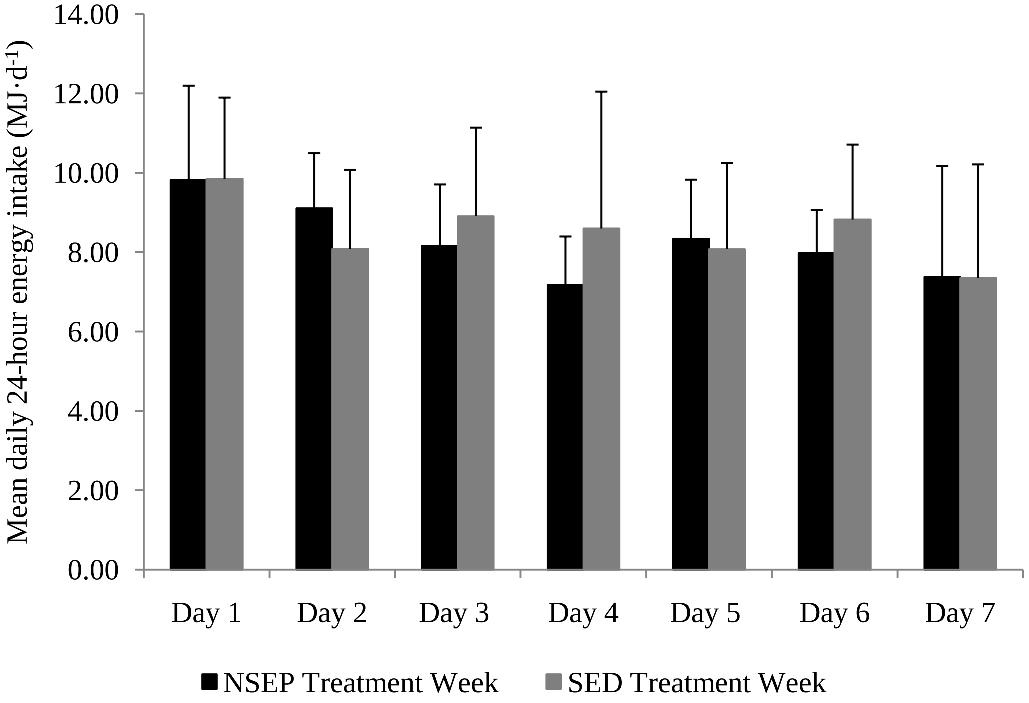


Figure 6.3 Mean ± SD total 5-day energy expenditure (MJ) for trained (n=5) and untrained (n=5) participants, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. *Significantly elevated in the NSEP treatment week compared to the SED treatment week (*p*=0.020)

6.3.2 Energy Intake

Mean ± SD daily 24-hour energy intake for each day in each treatment week, for trained and untrained participants is provided in figure 6.4a and 6.4b, respectively.

(a)



(b)

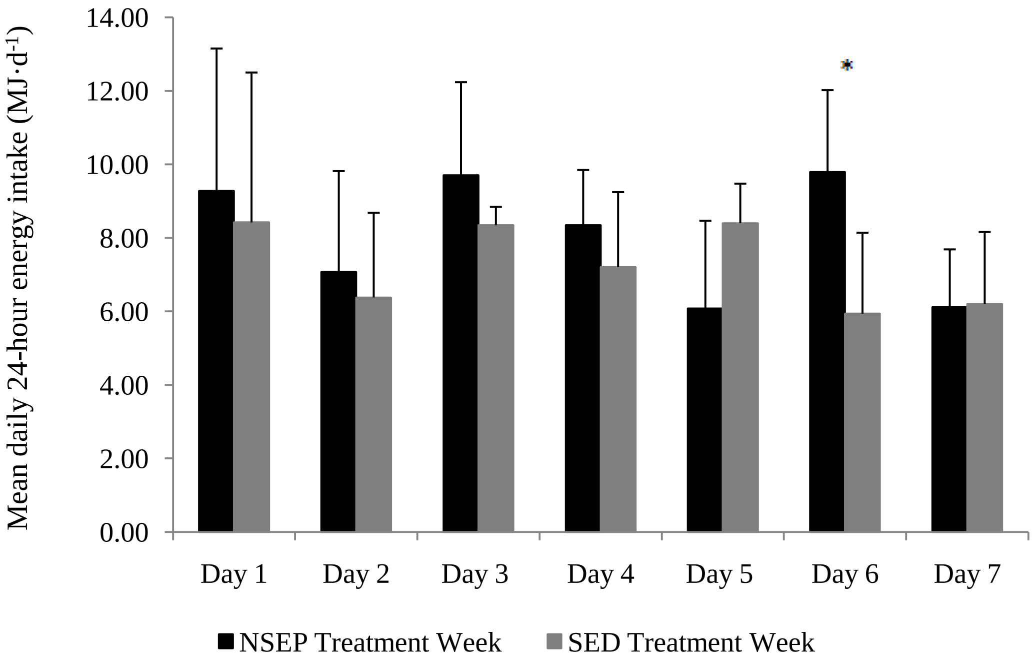


Figure 6.4. Mean ± SD daily 24-hour energy intake (MJ) for (a) trained (n=5) and (b) untrained participants (n=5), for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. *Significantly elevated on day 6 of the NSEP treatment week compared to day 6 in the SED treatment week in the untrained participants (*p*<0.05).

Days 1 and 2 (Maintenance days)

For daily 24-hour energy intake for the maintenance days 1 and 2, there were no significant main effects of intervention [$F(1,8)=1.537$, $p=0.250$] or day [$F(1,8)=2.635$, $p=0.143$] or interactions between intervention and training status [$F(1,8)=0.073$, $p=0.794$], week day and training status [$F(1,8)=0.179$, $p=0.684$] or intervention, week day and training status [$F(1,8)=1.254$, $p=0.295$].

Day 3 Test Meal (Intervention day)

For energy intake (MJ) at the test meal following the NSEP and SED interventions, for the trained and untrained participants, there were no significant main effects of intervention [$F(1,8)=0.219$, $p=0.652$] or interaction between intervention and training status [$F(1,8)=0.769$, $p=0.406$]. In the NSEP and SED interventions, the trained participants consumed 3.35±0.99 versus 3.28±0.88 MJ, respectively, and the untrained participants consumed 3.14±0.61 versus 3.39±1.06 MJ, respectively.

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

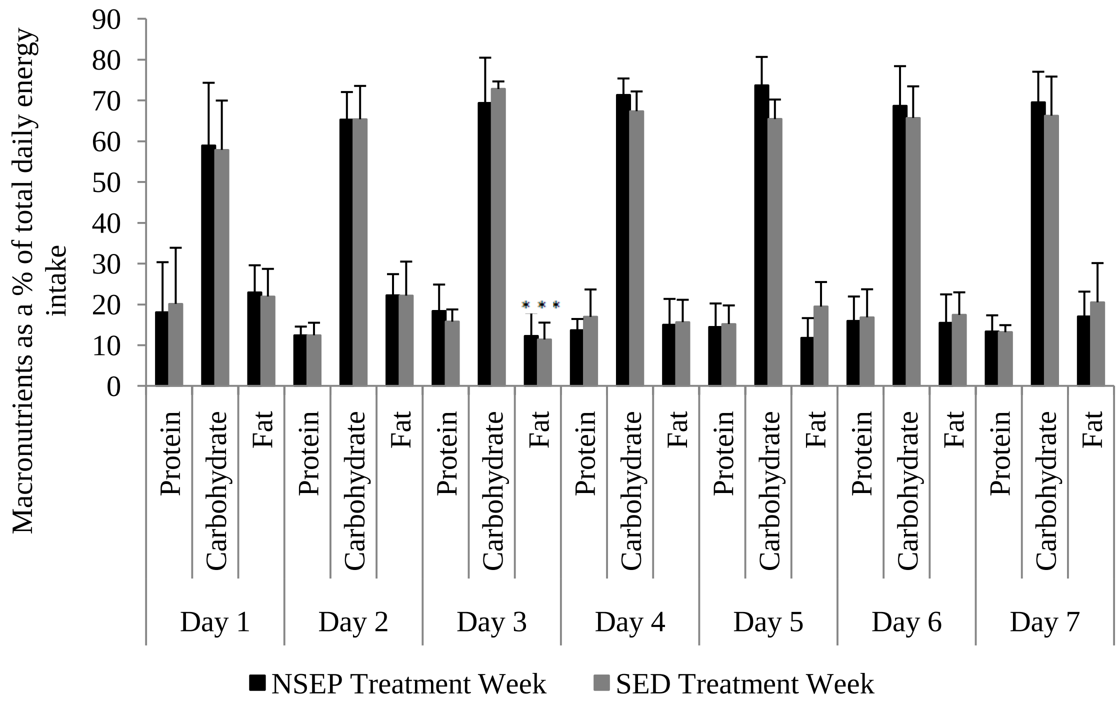
For day 3, 4, 5, 6 and 7 daily 24-hour energy intake, there was a significant main effect of week day [$F(4,32)=4.280$, $p=0.007$] and a significant interaction between intervention, week day and training status [$F(4,32)=3.793$, $p=0.012$]. Post-hoc Tukey tests identified that energy intake was significantly elevated on day 6 of the NSEP treatment week compared to the corresponding day in the SED treatment week ($p<0.05$), but only in the untrained girls, 9.79±2.22 versus 5.94±2.20 $MJ \cdot d^{-1}$, respectively.

For total 2-day, 3-day, 4-day and 5-day energy intake when the NSEP treatment week was compared to the SED treatment week, in the trained and untrained participants, no significant differences were identified.

6.3.3 Macronutrient Intake

Mean ± SD percentage of daily energy intake for all three macronutrients, protein, carbohydrate and fat, over days 1 to 7 in each treatment week, for trained and untrained participants are provided in figure 6.5a and 6.5b, respectively.

(a)



(b)

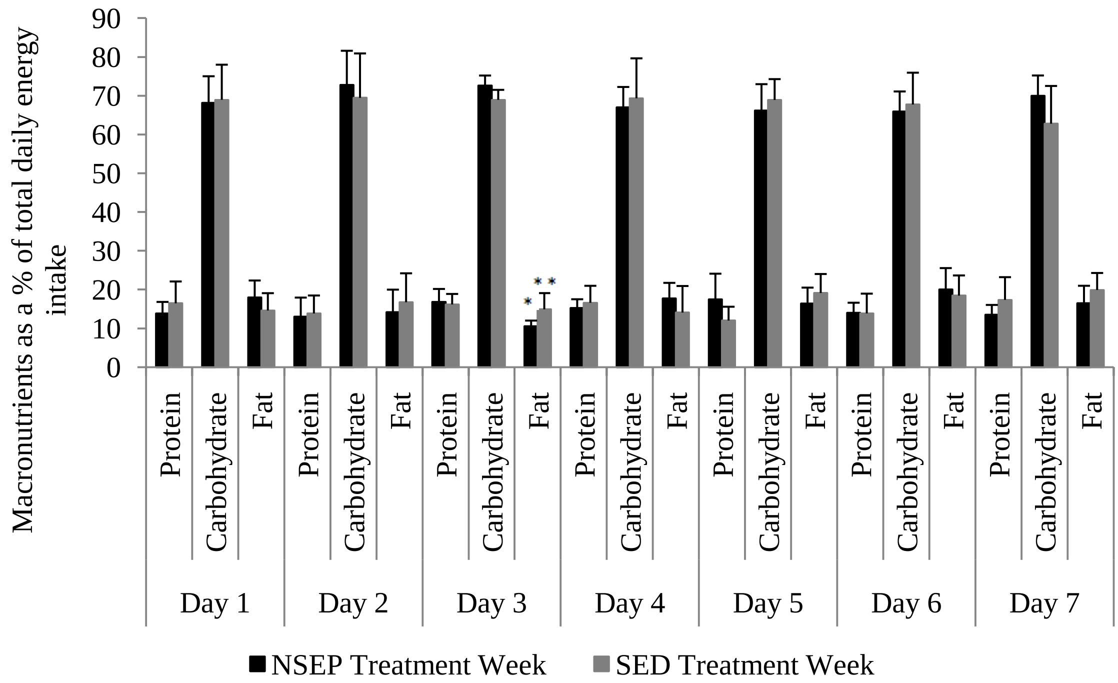


Figure 6.5. Mean ± SD macronutrient intakes as a percentage of total daily energy intake, for (a) trained (n=5) and (b) untrained (n=5) participants, for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. *Significantly lower compared to day 6 within the NSEP treatment week (*p*<0.05). **Significantly lower compared to day 5 (*p*<0.05) and day 7 (*p*<0.05) in the SED treatment week.

Days 1 and 2 (Maintenance days)

There were no significant main effects of intervention for protein [$F(1,8)=0.838$, $p=0.387$], carbohydrate [$F(1,8)=0.061$, $p=0.810$], or fat [$F(1,8)=0.043$, $p=0.841$], or main effect of week day for protein [$F(1,8)=2.000$, $p=0.195$], carbohydrate [$F(1,8)=1.640$, $p=0.236$], or fat [$F(1,8)=0.114$, $p=0.744$] for maintenance days 1 and 2. Similarily, there were no interactions between intervention and week day for protein [$F(1,8)=1.259$, $p=0.294$], carbohydrate [$F(1,8)=0.589$, $p=0.465$], or fat [$F(1,8)=1.785$, $p=0.218$], no interactions between intervention and training status for protein [$F(1,8)=0.045$, $p=0.838$], carbohydrate [$F(1,8)=0.012$, $p=0.916$], or fat [$F(1,8)=0.001$, $p=0.975$], no interactions between week day and training status protein [$F(1,8)=0.702$, $p=0.426$], carbohydrate [$F(1,8)=0.347$, $p=0.572$], or fat [$F(1,8)=0.033$, $p=0.861$], or between intervention, week day and training status for protein [$F(1,8)=0.003$, $p=0.954$], carbohydrate [$F(1,8)=1.717$, $p=0.226$], or fat [$F(1,8)=0.952$, $p=0.358$], for maintenance days 1 and 2.

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

For fat intake only on day 3, 4, 5, 6 and 7 significant main effects were found for intervention [$F(1,8)=14.412$, $p=0.005$] and week day [$F(4,32)=6.746$, $p<0.001$]. Post-hoc Tukey tests revealed that fat intake as a percentage of daily energy intake on day 3 of the NSEP treatment week was significantly lower compared to day 6 within the same week ($p<0.05$), 11.4±4.0 versus 17.8±6.4%, respectively. With regards to the SED treatment week fat intake as a percentage of daily energy intake on day 3 was significantly lower compared to day 5 ($p<0.05$) and day 7 ($p<0.05$) within the same week, 13.2±4.3 versus 19.3±5.2 and 20.2±7.0%, respectively. The significant difference found for intervention did not remain significant following post-hoc Tukey tests.

6.3.4 Energy Balance

Mean ± SD daily 24-hour energy balance for each day in each treatment week, for trained and untrained participants is provided in table 6.3.

Table 6.3. Mean ± SD daily 24-hour energy balance (MJ·d^{-1}) for each study day, for trained (n=5) and untrained participants (n=5), in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

	Trained participants (n=5)				Untrained participants (n=5)			
	NSEP		SED		NSEP		SED	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1 (MJ·d^{-1})	-0.87	1.82	-0.72	1.64	0.14	4.42	-0.65	4.46
Day 2 (MJ·d^{-1})	-1.20	1.77	-2.20	2.24	-1.79	2.95	-2.46	2.75
Day 3 (MJ·d^{-1})	-3.71	1.62	-1.96	2.24	-0.69	2.88	-0.97	0.55
Day 4 (MJ·d^{-1})	-3.58	0.78	-2.18	3.25	-0.76	1.66	-1.91	2.20
Day 5 (MJ·d^{-1})	-2.74	1.15	-2.69	1.79	-3.07	3.18	-0.74	1.67
Day 6 (MJ·d^{-1})	-2.70	0.68	-2.03	1.32	0.52	2.31	-3.17	2.75
Day 7 (MJ·d^{-1})	-3.37	2.51	-3.38	2.67	-3.12	1.46	-2.90	2.15
2-day (MJ)	-7.28	1.27	-4.13	5.22	-1.45	3.55*	-2.88	2.43
3-day (MJ)	-10.03	2.36	-6.82	6.97	-4.52	5.58	-3.61	3.63
4-day (MJ)	-12.73	2.71	-8.85	7.71	-4.00	6.87**	-6.78	6.35
5-day (MJ)	-16.10	5.07	-12.24	10.16	-7.12	6.62	-9.69	7.88

***Trend for the untrained girls to be in less of a negative energy balance compared to the trained girls, in the NSEP treatment week (*p*=0.063). **Trend for the untrained girls to be in less of a negative energy balance compared to the trained girls, in the NSEP treatment week (*p*=0.065).**

Days 1 and 2 (Maintenance days)

For daily 24-hour energy balance for the maintenance days 1 and 2, there were no significant main effects of intervention [$F(1,8)=1.373$, $p=0.275$], week day [$F(1,8)=1.786$, $p=0.218$], interaction between intervention and training status [$F(1,8)=0.097$, $p=0.764$], week day and training status [$F(1,8)=0.218$, $p=0.653$] or intervention and week day [$F(1,8)=1.043$, $p=0.337$] or between intervention, week day and training status [$F(1,8)=1.652$, $p=0.235$].

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

For day 3, 4, 5, 6 and 7 daily 24-hour energy balance, there was a significant interaction between intervention, week day and training status [$F(4,32)=3.119$, $p=0.028$]. However, when post-hoc Tukey tests were conducted these siginicant differences did not remain.

For total 2-day energy balance, there was a trend for the untrained girls to be in less of a negative energy balance compared to the trained girls, in the NSEP treatment week, -1.45±3.55 versus -7.28±1.27 MJ, respectively [$F(1,8)=4.658$, $p=0.063$]. Similarly, this was the case for 4-day energy balance, where there was also a trend for the untrained participants to be in less of a negative energy balance compared to the trained girls, in the NSEP treatment week, -4.00±6.87 versus -12.73±2.71 MJ [$F(1,8)=4.576$, $p=0.065$], respectively.

6.3.5 Subjective Appetite

Mean ± SD daily hunger, prospective food consumption and fullness ratings for days 1 to 7 are presented in table 6.4.

Table 6.4. Mean ± SD appetite ratings for hunger, prospective food consumption and fullness (mm), for trained (n=5) and untrained (n=5) participants, for each study day, in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

	Hunger (very hungry = 100mm)								Prospective food consumption (a lot = 100mm)								Fullness (very full = 100mm)							
	Trained				Untrained				Trained				Untrained				Trained				Untrained			
	NSEP		SED		NSEP		SED		NSEP		SED		NSEP		SED		NSEP		SED		NSEP		SED	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1	48	5	48	19	49	9	46	17	48	9	49	17	47	9	41	24	45	5	45	7	54	7	45	22
Day 2	49	9	54	14	50	16	39	9	51	10	53	15	45	13	40	13	50	4	45	5	49	7	50	10
Day 3	57	13	56	10	44	13	48	11	55	10	53	8	42	9	44	16	52	6	49	11	54	10	53	13
Day 4	51	18	54	14	46	12	48	14	51	17	52	11	42	14	43	16	43	10	44	13	55	14	56	8
Day 5	51	15	55	18	47	13	51	15	48	8	55	15	45	14	48	18	44	8	43	15	57	13	59	3
Day 6	55	15	54	20	53	27	54	17	54	10	50	15	48	25	48	15	48	8	40	16	65	13	56	7
Day 7	50	19	57	18	51	25	60	15	50	17	54	12	46	21	57	19	47	5	44	15	59	10	63	12

Days 1 and 2 (Maintenance days)

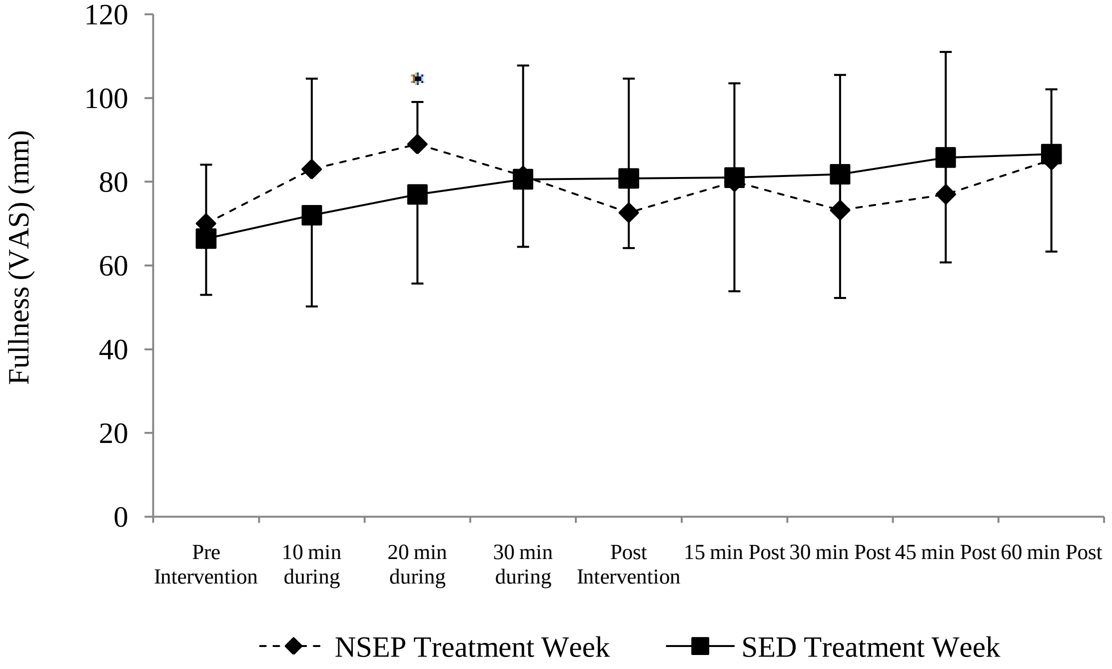
There were no significant main effects of intervention for hunger [$F(1,8)=0.462$, $p=0.516$], prospective food consumption [$F(1,8)=0.283$, $p=0.609$], or fullness [$F(1,8)=1.137$, $p=0.317$], or main effect of week day for hunger [$F(1,8)=0.10$, $p=0.923$], prospective food consumption [$F(1,8)=0.165$, $p=0.695$], or fullness [$F(1,8)=0.132$, $p=0.726$] for maintenance days 1 and 2. Similarily, there were no interactions between intervention and week day for hunger [$F(1,8)=0.111$, $p=0.748$], prospective food consumption [$F(1,8)=0.038$, $p=0.851$], or fullness [$F(1,8)=0.126$, $p=0.731$], no interactions between intervention and training status for hunger [$F(1,8)=2.171$, $p=0.179$], prospective food consumption [$F(1,8)=0.836$, $p=0.387$], or fullness [$F(1,8)=0.038$, $p=0.851$], no interactions between week day and training status hunger [$F(1,8)=2.531$, $p=0.150$], prospective food consumption [$F(1,8)=1.012$, $p=0.344$], or fullness [$F(1,8)=0.084$, $p=0.779$], or between intervention, week day and training status for hunger [$F(1,8)=1.149$, $p=0.315$], prospective food consumption [$F(1,8)=0.000$, $p=0.983$], or fullness [$F(1,8)=1.074$, $p=0.330$], for maintenance days 1 and 2.

Day 3 NSEP and SED Interventions

For hunger and prospective food consumption, assessed pre intervention, 10 minutes during, 20 minutes during, 30 minutes during, post intervention, 15 minutes post, 30 minutes post, 45 minutes post and 60 minutes post the NSEP and SED interventions, a significant main effect of time point was identified, [$F(8,64)=6.945$, $p=0.03$] and [$F(8,64)=7.491$, $p<0.001$], respectively.

Fullness values for pre intervention, 10 minutes during, 20 minutes during, 30 minutes during, post intervention, 15 minutes post, 30 minutes post, 45 minutes post and 60 minutes post the NSEP and SED interventions, for the trained participants, untrained participants and trained and untrained participants combined are provided in figures 6.6a, 6.6b and 6.6c, respectively.

(a)



(b)

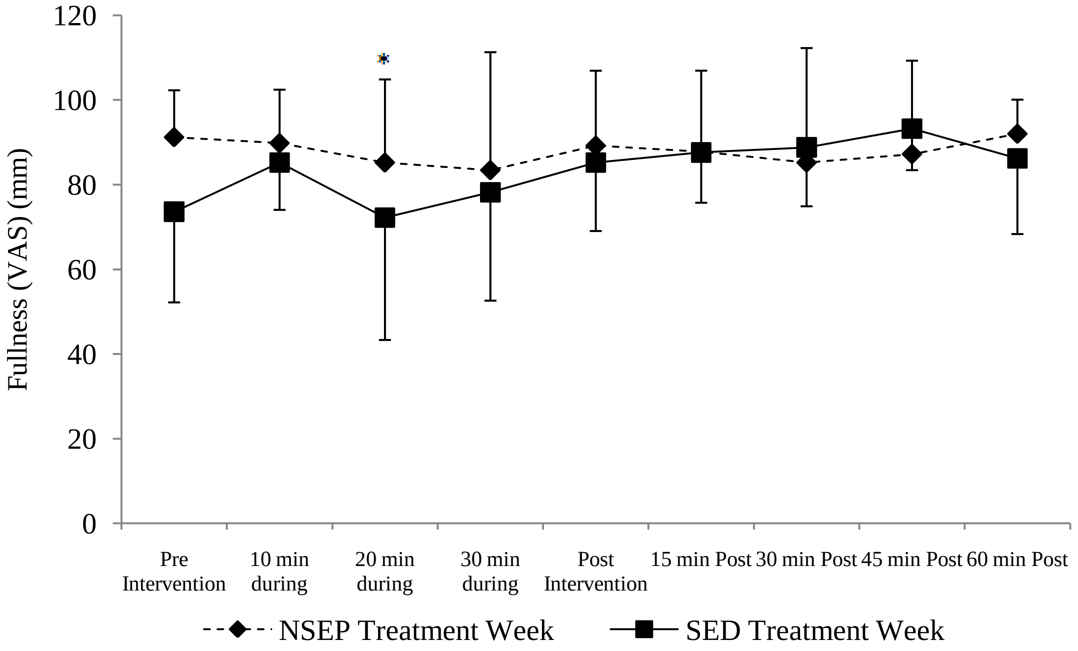


Figure 6.6. Mean ± SD fullness (mm) for (a) for trained participants (n=5) and (b) untrained participants (n=5), in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks. 100mm = very full. *Significantly different between interventions (*p*=0.026).

A significant interaction was identified between intervention and time point [F(8,64)=2.453, p=0.022]. Further one way repeated measures ANOVA identified that participants felt significantly more full 20 minutes during the NSEP intervention compared to the corresponding time point during the SED intervention, 87±15 versus 75±24 mm [F(1,8)=6.269, p=0.037, effect size = 1.18], respectively.

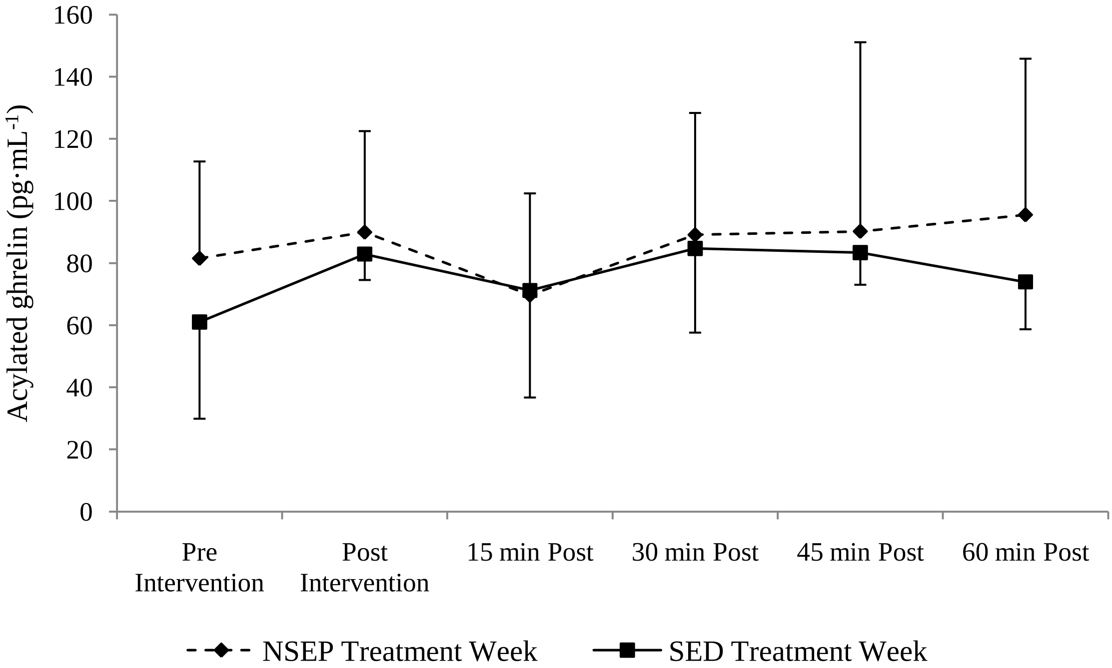
Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

There were no significant main effects of intervention for daily ratings of hunger [F(1,8)=3.413, p=0.102], prospective food consumption [F(1,8)=1.136, p=0.318], or fullness [F(1,8)=0.546, p=0.481], or main effect of week day for hunger [F(4,32)=0.917, p=0.466], prospective food consumption [F(4,32)=0.957, p=0.444], or fullness [F(4,32)=0.449, p=0.772] for days 3, 4, 5, 6 and 7. Similarily, there were no interactions between intervention and week day for hunger [F(4,32)=0.771, p=0.552], prospective food consumption [F(4,32)=1.164, p=0.345], or fullness [F(4,32)=0.822, p=0.521], no interactions between intervention and training status for hunger [F(1,8)=0.286, p=0.607], prospective food consumption [F(1,8)=0.361, p=0.565], or fullness [F(1,8)=0.224, p=0.648], no interactions between week day and training status hunger [F(4,32)=1.238, p=0.315], prospective food consumption [F(4,32)=1.334, p=0.279], or fullness [F(4,32)=1.720, p=0.170], or between intervention, week day and training status for hunger [F(4,32)=0.114, p=0.977], prospective food consumption [F(4,32)=0.331, p=0.855], or fullness [F(4,32)=0.165, p=0.955], for days 3, 4, 5, 6 and 7.

6.3.6 Acylated Ghrelin: NSEP and SED Interventions

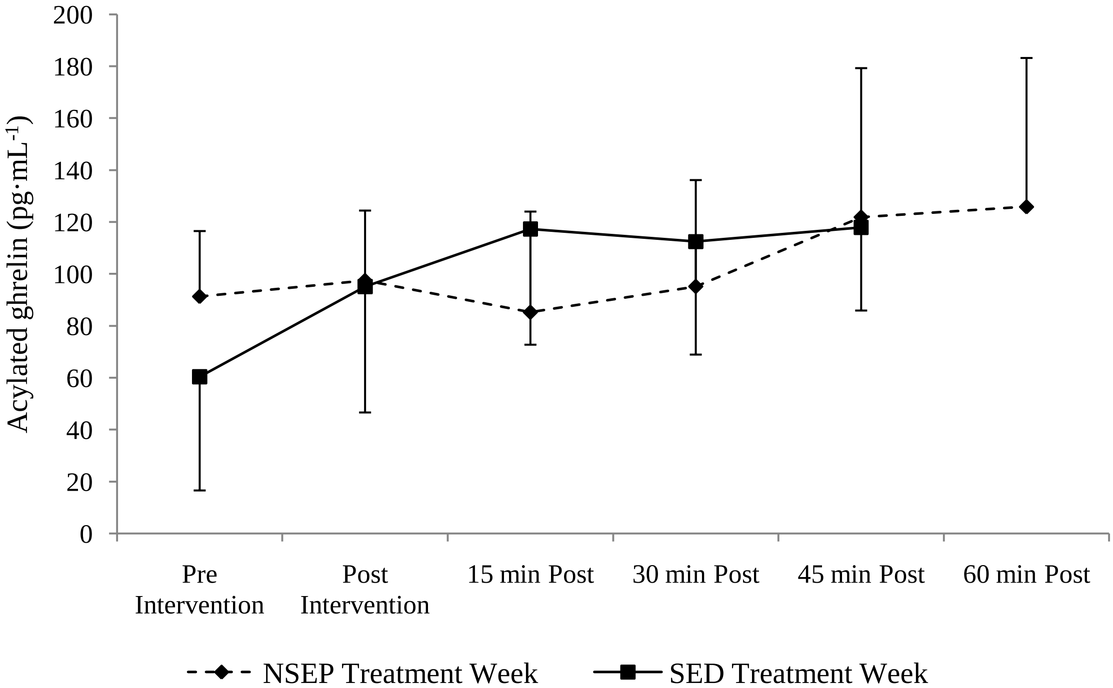
Mean ± SD plasma acylated ghrelin concentrations for pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post and 60 minutes post the NSEP and SED interventions for the trained participants are provided in figure 6.7a. Similiarly, for the untrained participants plasma acylated ghrelin concentrations are provided in figure 6.7b for the same time points, with the exception of the last time point (60 minutes post) in the SED intervention, due to assay difficulties. Figure 6.7c provides the plasma acylated ghrelin concentrations for all participants (n=7) for pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post and 60 minutes post the NSEP and SED interventions.

(a)



160
140
120
100
80
60
40
20
0
Acylated ghrelin (pg·mL-1)
Pre Intervention
Post Intervention
15 min Post
30 min Post
45 min Post
60 min Post
NSEP Treatment Week
SED Treatment Week

(b)



200
180
160
140
120
100
80
60
40
20
0
Acylated ghrelin (pg·mL-1)
Pre Intervention
Post Intervention
15 min Post
30 min Post
45 min Post
60 min Post
NSEP Treatment Week
SED Treatment Week

(c)

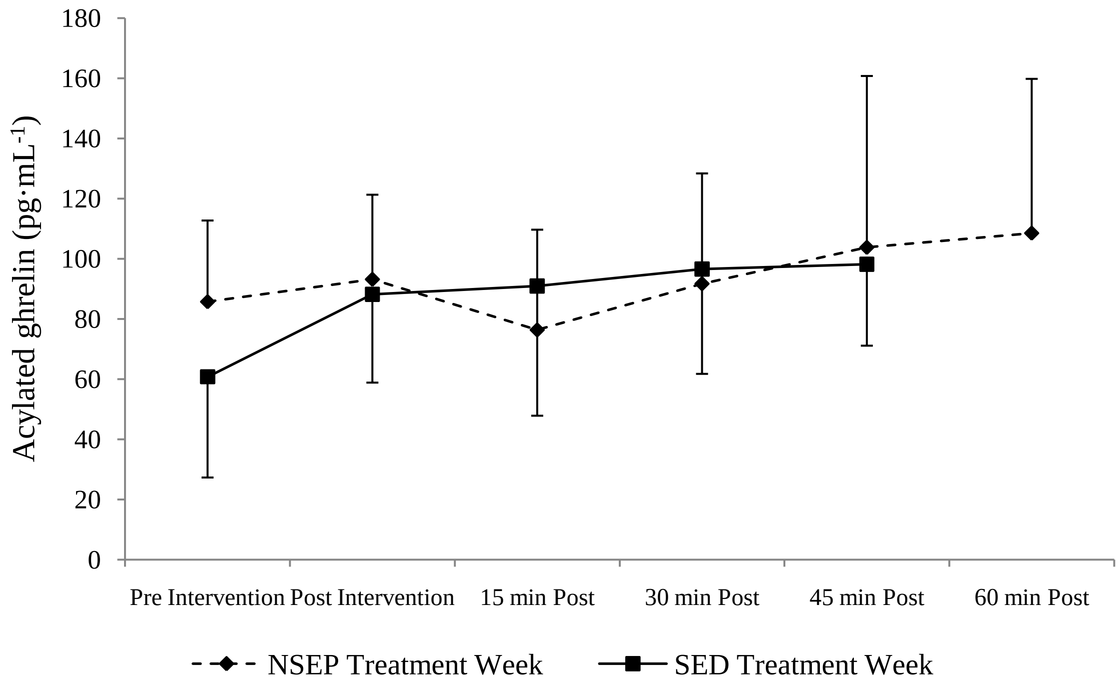


Figure 6.7. Mean ± SD plasma acylated ghrelin concentrations (pg·mL^{-1}) for (a) trained participants (n=4), (b) untrained participants (n=3) and (c) all participants (n=7), in the netball specific exercise protocol (NSEP) and sedentary (SED) treatment weeks.

Pre intervention plasma acylated ghrelin concentrations were not significantly different between the NSEP and SED interventions, for the trained participants (81.5±31.2 versus 61.0±31.1 pg·mL^{-1}, respectively), untrained participants (91.3±25.2 versus 60.4±43.7 pg·mL^{-1}, respectively) and all the participants as one group (85.7±27.0 versus 60.8±33.5 pg·mL^{-1}, respectively). These results were identified by the lack of interaction between intervention and time point in the trained participants [$F(5,15)=0.510$, $p=0.764$], untrained participants [$F(4,8)=1.434$, $p=0.307$] and the two groups as a whole [$F(4,24)=1.932$, $p=0.138$].

No significant main effects or interactions between intervention and time point were identified for the trained participants as a separate group. For the untrained participants however, with regards to plasma acylated ghrelin concentrations there was not a significant main effect of intervention [$F(1,2)=0.369$, $p=0.605$] or significant interation between intervention and time point [$F(4,8)$ =1.434, $p=0.307$], however, there was a significant main effect of time point [$F(4,8)=14.264$, $p=0.001$]. Post-hoc Tukey tests however could not be conducted due to the low sample size in the untrained participant group (n=3).

Therefore, plasma acylated ghrelin concentrations, for all the participants (trained, n=4; untrained, n=3) as a whole, found that there was not a main effect of intervention

[$F(1,6)=0.099$, $p=0.764$] or interation between intervention and time point [$F(4,24)=1.932$, $p=0.138$]. However, there was a trend for a main effect of time point [$F(4,24)=3.947$, $p=0.054$].

6.3.7 Correlations between Acylated Ghrelin and other Variables

Plasma acylated ghrelin concentrations prior to the first intervention were not significantly correlated with BMI, body mass, percentage body fat, maturity offset, baseline plasma insulin and baseline plasma glucose concentrations, in the untrained participants. However, significant correlations were found in the trained participants between baseline plasma acylated ghrelin and percentage body fat (r= 0.99, $p=0.007$, n=4) and also maturity offset (r= - 0.95, $p=0.047$, n=4).

Table 6.5 describes the correlations between plasma acylated ghrelin concentrations and subjective hunger, prospective food consumption, plasma insulin and glucose concentrations and test meal energy intake at individual time points (pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post, 60 minutes post) in both interventions, for both the trained and untrained participants. There were no significant correlations between any of the variables.

Therefore due to the low sample sizes in each group, table 6.6 was completed to describe the correlations between plasma acylated ghrelin concentrations and subjective hunger, prospective food consumption, plasma insulin and glucose concentrations and test meal energy intake at individual time points (pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post, 60 minutes post) in both interventions, for both the trained and untrained participants as one group (n=7). There were no significant correlations between any of the variables, although there was a trend for plasma acylated ghrelin concentrations in the SED intervention at 45 minutes post to be negatively correlated with prospective food consumption at the same time point (r= -0.74, $p=0.057$, n=7).

Table 6.5. Correlations (r values and *p* values) between plasma acylated ghrelin concentrations and subjective hunger, prospective food consumption (PFC), plasma insulin and glucose concentrations and test meal energy intake at individual time points (pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post, 60 minutes post) in both the netball specific exercise protocol (NSEP) and sedentary (SED) interventions, for both the trained (n=4) and untrained (n=3) participants.

Acylated Ghrelin		**NSEP Intervention**						**SED Intervention**					
		Pre	**Post**	**15 min Post**	**30 min Post**	**45 min Post**	**60 min Post**	**Pre**	**Post**	**15 min Post**	**30 min Post**	**45 min Post**	**60 min Post**
		r	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**
		p	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***
Hunger	**Trained**	-0.44	0.84	0.85	0.79	-0.09	0.33	-0.35	0.82	0.85	0.79	-0.64	-0.60
		0.559	0.158	0.149	0.213	0.915	0.671	0.650	0.182	0.147	0.211	0.362	0.400
	Untrained	-0.27	0.54	0.55	0.46	0.42	0.69	-0.27	0.57	0.45	0.62	0.13	
		0.827	0.635	0.627	0.696	0.724	0.514	0.827	0.614	0.703	0.577	0.919	
PFC	**Trained**	-0.81	0.76	0.92	0.38	-0.13	0.36	0.12	0.98	0.71	0.84	-0.67	0.32
		0.188	0.237	0.081	0.616	0.871	0.638	0.883	0.230	0.289	0.157	0.334	0.679
	Untrained	-0.45	0.56	0.59	0.27	0.48	-0.87	0.43	0.39	0.60	0.36	-0.38	
		0.702	0.620	0.601	0.829	0.678	0.334	0.715	0.746	0.591	0.765	0.753	
Insulin	**Trained**	-0.39	0.21	-0.64	-0.81	-0.88	-0.72	-0.71	0.46	0.80	0.69	0.16	0.19
		0.608	0.794	0.365	0.189	0.116	0.277	0.289	0.536	0.202	0.308	0.837	0.809
	Untrained	-0.60	-0.47	-0.74	-0.98	-0.06	-0.63	-0.83	-0.99	-0.98	-0.70	-0.30	
		0.589	0.688	0.475	0.133	0.965	0.567	0.382	0.081	0.139	0.507	0.806	
Glucose	**Trained**	-0.59	0.10	0.19	-0.21	-0.24	0.20	-0.52	0.06	-0.76	-0.47	-0.01	-0.31
		0.409	0.897	0.814	0.794	0.763	0.802	0.481	0.938	0.245	0.521	0.992	0.695
	Untrained	-0.76	0.03	-0.30	0.99	0.82	0.74	0.64	0.99	0.78	0.89	0.94	
		0.453	0.983	0.805	0.104	0.389	0.473	0.560	0.103	0.427	0.306	0.224	
Test Meal	**Trained**					-0.77						-0.76	
						0.214						0.236	
Energy Intake	**Untrained**					0.25						0.95	
						0.837						0.205	

Grey areas indicate missing blood samples, black areas indicate no data collection required. 45 minute post acylated ghrelin concentrations correlated with test meal energy intake in the two groups, in each intervention due to the missing samples for acylated ghrelin in the SED intervention (60 minutes post).

Table 6.6. Correlations (r values and *p* values) between plasma acylated ghrelin concentrations and subjective hunger, prospective food consumption (PFC), plasma insulin and glucose concentrations and test meal energy intake at individual time points (pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post, 60 minutes post) in both the netball specific exercise protocol (NSEP) and sedentary (SED) interventions, for the trained and untrained participants as a whole (n=7).

Acylated Ghrelin		**NSEP Intervention**						**SED Intervention**					
		Pre	**Post**	**15 min Post**	**30 min Post**	**45 min Post**	**60 min Post**	**Pre**	**Post**	**15 min Post**	**30 min Post**	**45 min Post**	**60 min Post**
		r	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**	**r**
		p	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***	***p***
Hunger	**All**	-0.24	0.51	0.47	0.45	0.12	0.36	-0.25	0.17	-0.21	0.06	-0.54	
		0.598	0.237	0.290	0.312	0.795	0.435	0.587	0.720	0.650	0.894	0.210	
PFC	**All**	-0.62	0.46	0.35	0.21	0.05	-0.11	0.08	0.06	-0.03	-0.18	-0.74*	
		0.139	0.296	0.448	0.645	0.918	0.819	0.864	0.908	0.944	0.707	0.057	
Insulin	**All**	-0.47	-0.13	-0.57	-0.44	-0.67	-0.69	-0.68	-0.43	-0.42	-0.17	-0.56	
		0.284	0.787	0.182	0.324	0.100	0.087	0.091	0.332	0.345	0.717	0.190	
Glucose	**All**	-0.38	0.15	0.18	0.17	0.06	0.48	-0.01	0.58	-0.38	0.17	0.05	
		0.405	0.746	0.695	0.720	0.902	0.274	0.991	0.176	0.402	0.718	0.916	
Test Meal	**All**					-0.51						0.25	
						0.243						0.594	

***Trend for plasma acylated ghrelin concentrations in the SED intervention at 45 minutes post to be negatively correlated with prospective food consumption at the same time point (*p*=0.057).**

6.3.8 Insulin and Glucose: NSEP and SED Interventions

Pre intervention plasma insulin concentrations were not significantly different between the NSEP and SED interventions in the trained participants (114.6±58.2 versus 124.2±18.0 pmol·L^{-1}, respectively), untrained participants (57.0±39.6 versus 93.6±80.4 pmol·L^{-1}, respectively) and the groups as a whole (90.0±56.4 versus 111±51 pmol·L^{-1}, respectively). This was identified by the lack of interaction between intervention and time point in the trained [F(5,15)=0.528, p=0.752], untrained [F(5,10)=1.544, p=0.261] and the groups as a whole [F(5,30)=1.159, p=0.352]. When examining individual time points (pre intervention, post intervention, 15 minutes post, 30 minutes post, 45 minutes post, 60 minutes post) there was a significant main effect of time point [F(5,25)=5.754, p=0.001].

Similarly, pre intervention plasma glucose concentrations were not significantly different between the NSEP and SED interventions in the trained participants (3.8±0.5 versus 4.6±0.5 mmol·L^{-1}, respectively), untrained participants (4.4±0.4 versus 4.3±0.5 mmol·L^{-1}, respectively) and the groups as a whole (4.1±0.5 versus 4.5±0.5 mmol·L^{-1}, respectively). This was identified by the lack of interaction between intervention and time point in the trained [F(1.740, 5.219)=3.400, p=0.116] and untrained [F(1.194, 2.388)=3.403, p=0.190] participants, although as a whole group an interaction between intervention and time was identified [F(5,30)=6.699, p<0.001].

6.3.9 Exercise Intensity

In the trained participants, the self-paced NSEP induced an average exercise intensity of 69±8% of $\dot{V}O_{2\ peak}$, with the participants working on average at 79±5% of maximum heart rate. In the untrained participants, the self-paced NSEP induced a slightly higher average exercise intensity of 75±9% of $\dot{V}O_{2\ peak}$, with the untrained participants working at 83±6% of maximum heart rate, which was slightly higher compared to the trained participants. There was no significant difference between the trained and untrained participants for percentage $\dot{V}O_{2\ peak}$ [F(1,8)=1.378, p=0.274] during the NSEP.

6.3.10 Ratings of Perceived Exertion and Heart Rate

Mean ± SD ratings of perceived exertion values (using the Pictorial Children's Effort Rating Table) and heart rate sampled immediately before and after the NSEP intervention and after each 10 minute quarter during the NSEP intervention, are described in table 6.7.

Table 6.7. Mean ± SD ratings of perceived exertion using the Pictorials Children's Effort Rating Table (P-CERT) and heart rate (b·min^{-1}), for trained (n=5) and untrained participants (n=5), in the netball specific exercise protocol (NSEP).

	Trained participants (n=5)				Untrained participants (n=5)			
	P-CERT		Heart rate (b·min^{-1})		P-CERT		Heart rate (b·min^{-1})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre NSEP	1	0	86	11	1	0	85	13
10 mins into NSEP	4	1	145	15	5	2	170	17*
20 mins into NSEP	6	2	155	11	6	1	162	19
30 mins into NSEP	7	2	160	14	7	0	166	16
Post NSEP	7	1	155	11	8	1	170	15

***Significantly higher in the untrained participants compared to the trained participants (F(1,8)=5.959, p=0.04).**

6.4 Discussion

The present study was the first intervention study to examine acylated ghrelin responses to exercise in trained and untrained adolescent girls, alongside free-living 24-hour energy intake, macronutrient intake, 24-hour energy expenditure, 24-hour energy balance and subjective appetite (hunger, prospective food consumption, fullness). The main findings of this study were that the untrained girls increased their energy intake on day 6 of the NSEP treatment week and therefore were in a positive energy balance. In addition, the exercise-induced energy expenditure on day 3 in the NSEP treatment week resulted in total 5-day energy expenditure being elevated in the trained and untrained participants when compared to the SED treatment week. With regards to subjective appetite, both the trained and untrained girls felt fuller 20 minutes into the NSEP intervention in comparison to the corresponding time during the SED intervention. Finally, in terms of plasma acylated ghrelin levels, a significant positive relationship was identified between ghrelin and maturity offset and a significant negative relationship identified between ghrelin and percentage body fat in the trained girls.

6.4.1 Protocol Considerations

The present study was an extension of the previous study in this thesis, therefore section 5.4.1 can be referred to for issues discussed previously regarding protocol considerations which also apply to this study. There were no significant differences for 24-hour energy expenditure, 24-hour energy intake, 24-hour energy balance and daily hunger, prospective food consumption and fullness ratings for maintenance days 1 and 2. This suggests that the girls began one treatment week at the same relative point to that of the previous week (Stubbs *et al.*, 2004). Throughout the following discussion reasons for significant and nonsignificant findings are discussed, however the low number of participants due to the demands and logistics of the research must be considered (trained, n=5; untrained, n=5). Having only five participants in each group may have limited the meaningfulness of the statitiscal analyses, but this was a very complex study, which relied a lot on how the adolescent girls coped with the cannulation procedure, since this was the first study to explore plasma acylated ghrelin concentrations following exercise in this population.

6.4.2 Energy Expenditure

With regards to daily energy expenditure over all 7-days, energy expenditure on the intervention day (day 3) and average exercise-induced energy expenditure, no differences were identified between the trained and untrained girls. As intended, energy expenditure was only elevated on day 3 of the NSEP treatment week when compared to all other days within that week and the corresponding day 3 in the SED treatment week, in both the trained (11.87±0.69 versus 10.86±0.60 $MJ \cdot d^{-1}$) and untrained girls (10.40±1.12 versus 9.31±0.85 $MJ \cdot d^{-1}$), respectively.

Interestingly, however, unlike the findings in the previous study of this thesis (chapter five) the elevated energy expenditure on day 3 of the NSEP treatment week did have an overall effect on total 5-day energy expenditure when the treatment weeks were compared, in the trained and untrained girls. This difference may be due to a more sensitive technique for assessing energy expenditure, in the form of combined heart rate and accelerometry being employed in the present study. Unlike using heart rate to predict energy expenditure, the combination of heart rate and activity data allows researchers estimate energy expenditure more accurately (Brage *et al*., 2004). This finding is in line with previous paediatric findings, where Dodd, Welsman and Armstrong (2008) identified an elevated energy expenditure over 5-days during an exercise week, in a group of young girls (10-11 years old) when compared to a sedentary week.

Similar to in chapter five, it was not the aim of this study to impose a specific exercise intensity at which the girls were required to exercise at, but rather to quantify what intensity the NSEP induced, taking into account it was designed as a self-paced intermittent replica netball protocol for the respective age group. With regards to exercise intensity as a percentage of $\dot{V}O_{2\ peak}$ and maximum heart and ratings of perceived exertion assessed using the Pictorial Children's Effort Rating Table (Yelling, Lamb & Swaine, 2002), during the NSEP intervention, there were no differences between the trained and untrained girls. Ratings of perceived exertion and heart rate values were however comparable to those identified in chapter five of this thesis. During the NSEP intervention the trained participants were exercising at 69±8% of $\dot{V}O_{2\,peak}$ and a heart rate of 79±5% with regards to maximum heart rate. The untrained participants were exercising at 75±9% of $\dot{V}O_{2\,peak}$ and a heart rate of 83±6% with regards to maximum heart rate. In chapter five of this thesis the values for exercise intensity and maximum heart rate equated to 64±5% $\dot{V}O_{2\,peak}$ and 83±6% of maximum heart rate.

6.4.3 Energy Intake and 24-hour Energy Balance

In the trained and untrained girls, following the 47 minute NSEP intervention, energy intake at the test meal was not elevated compared to energy intake following the SED intervention. This lack of energy intake compensation at a test meal following a bout of exercise has previously been identified in trained adolescent girls (12-14 years) completing the same netball intervention (Rumbold & Dodd, 2007).

However, over 3-days (72-hours) following the NSEP intervention, an elevation in energy intake, in the untrained girls on day 6 in the NSEP treatment week when compared to the corresponding day in the SED treatment week was identified. With regards to all the other study days (days 3, 4, 5, 6 and 7) no differences in 24-hour energy intake were found, either between or within treatment weeks or between the trained and untrained girls. An elevation in energy intake has been identified in paediatric groups, in the previous study of this thesis, where trained adolescent girls elevated their energy intake over 2-days (48-hours) following the same NSEP intervention when compared to the corresponding time period following a SED intervention. Initially, in line with the results of the previous study of this thesis, it was hypothesised in the present study, that compared to untrained adolescent girls, trained adolescent girls may be better able to regulate their energy intake in response to an elevation in energy expenditure resulting from exercise. This therefore would correspond with findings in adults where habitual exercisers have demonstrated an ability to regulate their energy intake compensation in response to previous dietary energy intake following exercise (King *et al*., 1999; Long, Hart & Morgan, 2002). However, the findings of the present study contradict this.

Instead an elevation in energy intake in the untrained girls on day 6 in the NSEP treatment week resulted in them being in a positive energy balance, and although post-hoc analysis did not reveal any significant differences between interventions, week days or training status, the untrained girls were always in less of a negative energy balance in comparison to the trained girls. There was however a lot of day-to-day variability in energy intake in the untrained participants compared to the trained. Indeed, there was a trend for the untrained girls to be in less of a negative energy balance compared to the trained girls, in the NSEP treatment week, with regards to total 2-day energy balance and total 4-day energy balance. A possible explanation as to why there was a difference in energy balance status between the trained and untrained girls, may have been due to the trained girls having higher resting metabolic rate values (approaching significance) compared to the untrained girls. With regards to lean body mass, which is a major component of resting metabolic rate, training has been associated with an increase in lean body mass in adolescent females (Malina, Bouchard & Bar-Or, 2004). Thus it has been suggested that energy expenditure is highly influenced by not only the size of the fat-free mass but also the composition (Reilly, 1998). Although not significantly different, the trained girls in the present study tended to have higher 24-hour energy expenditure values compared to the untrained girls, thus providing a possible explanation for the trained girls being in more of a negative energy balance in comparison to their untrained peers.

6.4.4 Macronutrient Intake

There was no difference in macronutrient intake between the trained and untrained girls over 7-days in the present study. An initial reason may be the low number of participants involved in the present study (trained, n=5; untrained, n=5) due to the demands and logistics of the research. In the previous study of this thesis it was suggested that trained adolescent populations in comparison to untrained adolescent populations may require an elevated intake of carbohydrate (55-60%) as part of their habitual daily energy intake in order to meet the nutritional demands of physical activity and health (A.D.A, 1996). This has been shown not to be the case in the present study, suggesting that unlike trained adults, trained adolescent groups may not necessarily need to consume higher amounts of carbohydrate compared to their untrained counterparts. An explanation for this may be due to the exercise behaviour of young people in general being sporadic (Fawkner & Armstrong, 2007). Netball is described as an interval sport, involving short sprints followed by recovery periods (Allison, 1978), which may also be characteristic of young peoples general activity patterns. Consequently, all girls, regardless of being trained or untrained will require energy primarily from carbohydrate rich sources in comparison to fat, to continually replenish liver and muscle glycogen stores, enabling subsequent bouts of moderate to high intensity exercise to be completed (McArdle, Katch & Katch, 1991).

Interestingly, percentage fat on the intervention day (day 3) in the NSEP and SED treatment weeks for the trained and untrained participants, when the test meal was provided, was significantly lower compared to the proportion of fat (%) consumed on all the other days in the treatment weeks (maintenance day 1 and 2, follow up days 4, 5, 6 and

7). The macronutrient content of the test meal provided was 76.5% carbohydrate, 16.5% protein and 7.0% fat. Therefore the test meal may have had an overall effect on the macronutrient intake on day 3 of each of the treatment weeks.

6.4.5 Subjective Appetite

With regards to the study days 3, 4, 5, 6 and 7 there were no significant differences in 24-hour hunger, prospective food consumption or fullness ratings, either between or within treatment weeks or between trained and untrained girls. There was also no difference in mean hunger, prospective food consumption and fullness immediately following the NSEP and SED interventions, which with regards to hunger does not correspond with findings from the previous study of this thesis. However, appetite findings from the present study have previously been identified in 12-14 year old habitually active girls after completing a single bout of NSEP (Rumbold & Dodd, 2007), where similarily no change in subjective appetite was identified.

The main finding of the present work with regards to subjective appetite, was that both the trained and untrained girls felt significantly more full 20 minutes during the NSEP compared to the corresponding time in the SED intervention. Although these results do not correspond with the paediatric appetite literature, they do relate to findings from the adult literature that suggests hunger is suppressed during high intensity in females (King *et al*., 1996). It may be the case that children and adolescents use the terms ‘fuller’ and ‘could eat less’ interchangeably to convey feelings of not being hungry during exercise or a suppression in hunger which is inherently identified in adult males. Appetite responses to exercise with regards to paediatric groups are inconsistent, therefore emphasising the need for an objective measure of appetite to be investigated, such as hormone analysis in the form of acylated ghrelin.

With regards to the effect of the intervention (NSEP versus SED) on acylated ghrelin concentrations, no significant difference were identified in the trained and untrained participants, possibly due to the low participant numbers (trained n=4; untrained n=3). In the untrained girls, there was however a reduction in the acylated ghrelin concentrations at 15 minutes post the NSEP intervention compared to 15 minutes post the SED intervention, which then gradually increased so that at 45 minutes post the NSEP intervention, the acylated ghrelin concentrations corresponded with concentrations in the SED intervention at the same time point. This potentially corresponds with the notion that subjective appetite is suppressed for up to 15 minutes after exercise primarily in adult males (Blundell & King, 1999), although this has not been consistently identified using subjective measures of appetite (VAS) in paediatric populations (Moore *et al*., 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008). The present study did identify the potential for prospective food consumption 45 minutes post a sedentary condition to be negatively correlated with acylated ghrelin concentrations at the same time point in the corresponding intervention. The correlation was conducted on seven participants, therefore it is felt that all the correlations conducted in the present need to be completed with a larger sample size.

6.4.6 Acylated Ghrelin Responses to Exercise

Previous to this present thesis no research had been conducted into acylated ghrelin responses to exercise in adolescent girls. Pre intervention plasma acylated ghrelin concentrations were not significantly different between the NSEP and SED interventions, thus the replication of energy intake between maintenance days 1 and 2 and on the morning of both interventions was successful, in the four trained and three untrained girls. The reason for the alteration in the participant numbers, with regards to the blood analysis was due to difficulties obtaining full blood samples at the required time points for one of the trained girls and two of the untrained girls.

The present study did not identify a change in acylated ghrelin concentrations following the NSEP intervention in the four trained girls. For plasma acylated ghrelin concentrations, in the untrained participants, there was a significant main effect of time point (p=0.001), however post-hoc Tukey tests could not be conducted due to the low sample size in the untrained participant group (n=3). When plasma acylated ghrelin concentrations were investigated for all time points between the NSEP and SED interventions, for all the participants (trained, n=4; untrained, n=3) as a whole (n=7), it was identified that there was only a trend for a main effect of time point (p=0.054). Therefore post-hoc testing could not be conducted to determine the location of this potential significance.

With regards to the direct comparison of the NSEP and SED interventions in terms of acylated ghrelin concentrations, as mentioned previously (see section 6.4.5), due to the low participant numbers in the trained (n=4) and untrained (n=3) groups, there was no significant differences between the interventions identified. However, in the untrained participants, there was a reduction in the acylated ghrelin concentrations at 15 minutes post the NSEP intervention compared to 15 minutes post the SED intervention, which then gradually increased so that at 45 minutes post the NSEP intervention, the acylated ghrelin concentrations corresponded with concentrations in the SED intervention at the same time point. Two studies investigating acylated ghrelin responses to exercise, also identified a decrease in ghrelin levels in nine habitually active (soccer, rugby, tennis and hockey) males (19-25 years old) following 60 minutes of treadmill running at 75% $\dot{V}O_{2\,max}$ (Broom *et al.*, 2007) and in nine healthy males (18-27 years) during and immediately following 90 minutes of treadmill running at 70% $\dot{V}O_{2\,max}$ (King *et al.*, 2010). In the present study the trained and untrained adolescent girls were exercising at a similar intensity (69±8% $\dot{V}O_{2\,peak}$), to that of the Broom et al. (2007) study (75±9% $\dot{V}O_{2\,max}$) and King et al. (2010) study (70% $\dot{V}O_{2\,max}$). Comparing adult and paediatric data directly presents limitations, however with regards to acylated ghrelin responses to exercise. To my knowledge these adult studies are two of the first to be conducted and thus comparisons are inevitable. Major differences between the present study and that of Broom et al. (2007) and King et al. (2010), in addition to the age of the participants, are the mode and duration of the exercise and gender. The NSEP intervention used in the present study was intermittent in nature lasting 40 minutes in comparison to the 60 minutes of continuous running exercise used in the study by Broom et al. (2007) and 90 minutes by King et al. (2010). With regards to gender, there are inherent differences in subjective appetite responses to exercise in males and females, with males experiencing a suppression in hunger immediately following exercise (Thompson, Wolfe & Eikelboom, 1988; King, Burley & Blundell, 1994; King & Blundell, 1995) and females not (King *et al.*, 1996). Interestingly this suppression in subjective hunger is not dependent on the mode of exercise (King & Blundell, 1995), however it is dependent on the intensity and duration of the exercise bout (Thompson, Wolfe & Eikelboom, 1988; King, Burley & Blundell, 1994) in males. Since exercise intensity was comparable between the present study and that of Broom et al. (2007) and King et al. (2010), it may be that exercise duration (60 and 90 minutes versus 47 minutes in the present study) and or mode of exercise (continuous cycling versus intermittent netball exercise in the present study) influences acylated ghrelin release following exercise. An additional explanation could also be that changes in acylated ghrelin responses (an objective measure of appetite) to exercise follow the same pattern as subjective hunger. Thus a suppression in subjective hunger and potentially objective hunger (acylated ghrelin) may be exclusive to males and therefore will not be consistently identified in females. This may explain why there was no change in acylated ghrelin concentrations following exercise in the present study. Interestingly, however King et al. (1996) identified a significant suppression of subjective hunger actually during a bout of high intensity (70% $\dot{V}O_{2\,max}$), long duration (50 minutes) cycling exercise in 13 lean, unrestrained females

(22.6±2.3 years) (*p*<0.05). With regards to the present study, it was extremely logistically demanding to collect the required blood samples at the designated time points with regards to the NSEP and SED interventions (immediately before and after the interventions). Therefore, having had collected an additional blood sample during the intervention periods would not have been logistically possible.

It must also be acknowledged that the baseline characteristics for acylated ghrelin in adolescent girls are unknown, since this is the first study, to our knowledge, to explore acylated ghrelin following sport-specific exercise in this population group. The lack of data in terms of acylated ghrelin maybe to do issues regarding the cannulation procedure and its use with adolescent populations. However, there is also limited exercise and appetite data without the analysis of acylated ghrelin. Indeed, the only available baseline data is that of adult males (Broom *et al*., 2007; King *et al*., 2010) and therefore comparisons can be made. In the present study, acylated ghrelin levels were (mean±SD) 85.7±27.0 and 60.8±33.5 pg·mL^{-1}, before the NSEP and SED interventions, respectively and in the study of King et al. (2010) values (mean±SEM) were 147.1±19.7 and 130.3±15.1 pg·mL^{-1} before the control and exercise condition, respectively. Differences apparent may be attributable to factors such as age, gender, and most importantly whether participants were preprandial or postprandial. At present it is unknown how these factors may influence acylated ghrelin concentrations, especially food intake status. In the present study participants were postprandial when the baseline acylated ghrelin samples were taken, which may explain why the acylated ghrelin concentrations were lower than those in the study by King et al. (2010), where participants were fasted prior to the baseline blood collection. Further research in this field is therefore warranted.

6.4.7 Acylated Ghrelin and Correlation with other Variables

In the present study, plasma acylated ghrelin concentrations were not correlated with plasma glucose and insulin concentrations in the trained and untrained adolescent girls. Broom et al. (2007) identified similar findings in untrained male participants.

An interesting finding from the present study was that plasma acylated ghrelin concentrations were positively correlated with percentage body fat (r= 0.99) and negatively correlated with maturity offset (r= -0.95), in the trained adolescent girls only. These findings contradict those previously identified in 25 lean, trained adolescent girls (Jürimäe *et al*., 2007a), where plasma ghrelin concentrations were negatively correlated with percentage body fat. An important difference between these studies however is that total ghrelin was sampled by Jürimäe et al. (2007a), whereas acylated ghrelin, which is essential for ghrelin biological activity (Kojima *et al*., 2001) was sampled in the present study. Ghrelin has been implicated as a long-term regulator of energy balance (Tschöp, Castañeda & Pagotto, 2004). Despite the short half life of ghrelin (5-15 minutes), chronic administration does induce a positive energy balance (Tschöp, Castañeda & Pagotto, 2004). Although counterintuitive, circulating ghrelin levels are low in obese individuals, since ghrelin levels decrease in response to overfeeding (Neary, Goldstone & Bloom, 2004). Ghrelin levels increase in response to a chronic negative energy balance, which can be exercise related (Ravussin *et al*., 2001; Neary, Goldstone & Bloom, 2004). Although findings are for adults, the diurnal pattern of ghrelin over the short and long term does suggest that ghrelin is sensitive to changes in energy flux (King *et al*., 2010). Particularly in response to diet and exercise-induced energy restriction, which can lead to elevated ghrelin concentrations during the night-time period and when feeding at meals is initiated (Leidy *et al*., 2007). Thus there are few explanations for the positive relationship between acylated ghrelin and percentage body fat. It may be the case that the lower percentage body fat levels as a result of regular training, suppress acylated ghrelin thus promoting a negative energy balance. However, further research needs to be conducted to establish whether there is a genuine relationship between acylated ghrelin and percentage body fat in adolescent populations. Findings from the present study with regards to ghrelin concentrations and maturity, however do correspond with those of Jürimäe et al. (2007a), in that ghrelin was negatively correlated with a measure of sexual maturity (estradiol) in the trained adolescent girls. With increasing age a decrease in ghrelin concentrations has been identified (Whatmore *et al*., 2003), which could explain the negative relationship identified between maturity offset (somatic indicator of maturity based on biological age) and acylated ghrelin, in the present study. With regards to maturation status, the trained girls in the present study all had a positive maturity offset value. Indeed, the initiation of puberty is associated with a decrease in plasma ghrelin concentrations (Whatmore *et al*., 2003), thus explaining the negative relationship between ghrelin and maturity offset. The small sample sizes of the groups (trained, n=5; untrained, n=5) used in the present study must be considered, since this is a limiting factor of the research.

6.4.8 Trained versus Untrained

The habitual daily activity levels were significantly higher in the trained adolescent girls compared to the untrained adolescent girls in the present study, demonstrated by the daily physical activity counts in preliminary week 1 prior (see section 6.2.4) to the interventions, 5894.1±829.0 and 4920.2±1001.5 ($1\cdot day^{-1}$), respectively. Unlike adults who can easily be categorized as trained and untrained, it may be more difficult to group adolescents in this way. Firstly, the exercise behaviour, in the form of free play, house chores and school physical education, of young people in general is sporadic (Fawkner & Armstrong, 2007), similar to the nature of netball, which is described as an interval sport, involving short sprints followed by recovery periods (Allison, 1978). For the purpose of this thesis, adolescent girls who only participated in the forms of physical activity previously identified were classified as 'untrained' as opposed to training for a specific sport which then they would be classified as 'trained'.

6.4.9 Conclusion

In conclusion, the present study demonstrated that, untrained 13-15 year old girls undergoing intermittent netball exercise, increased their free-living energy intake 3-days (72-hours) following a bout of netball exercise compared to a sedentary intervention. The similarity between findings in chapter five and six of this thesis, in terms of the elevation in free-living energy intake 2-3 days following an exercise bout, implies that the monitoring of free-living energy intake may be the influential factor which may facilitate the identification of a compensatory increase in energy intake, regardless of training status.

In addition, both trained and untrained girls reported that they felt significantly more full 20 minutes during the netball exercise compared to the corresponding time in the sedentary intervention. There was a trend for acylated ghrelin concentrations to be lower (although not significant) 15 minutes following the netball exercise compared to 15 minutes following the sedentary intervention in the group as a whole and specifically in the untrained adolescent girls. The implications of these findings, together, suggest that intermittent exercise may possibly play a role in suppressing appetite in adolescent girls, indeed more so in untrained populations. The small sample size in the present study is acknowledged and it is recognised that such research is in its infancy and therefore this area requires further exploration.

However, this was the first study to intervene with an exercise protocol in order to explore acylated ghrelin levels alongside energy intake and subjective appetite in trained and untrained adolescent girls, identifying a disassociation between subjective and objective cues of hunger and changes in appetite. The majority of adult studies which have explored ghrelin concentrations following exercise have used total ghrlein as opposed to acylated ghrelin. In addition, this study also explored 24-hour energy intake (using the combined food diary and 24-hour recall method), 24-hour energy expenditure (using heart rate and accelerometry), 24-hour energy expenditure and subjective appetite. Therefore there was high demand placed on the adolescent girls who participated in the study, both in terms of the testing weeks and the preliminary testing week (measurements of body composition, maturity offset etc). Therefore due to the complexity (cannulation procedure to collect acylated ghrelin samples) and length (4 weeks) of the study, recruitment of young people willing to participate in such research is likely to be influenced. Therefore, these issues may have reduced the ability to detect significant differences between the variables examined in the study. With regards to the acylated ghrelin assay, it has taken a long time to perfect the technique, whilst the actual time period for the assay is also lengthy (overnight), therefore without the help of Broom et al. (2007) with regards to the assay procedures, this study would not have been impossible.

CHAPTER 7

General Conclusion

The main aim of this thesis was to explore free-living energy intake and appetite (subjective and objective) following sport-specific exercise in adolescent girls (chapters five and six). The two paediatric studies available in this domain have been laboratory-based in terms of the exercise bout which was imposed (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008). Therefore, a second aim of this thesis was to develop a sport-specific fitness test and exercise bout which was representative of the activity patterns of the sample population and therefore could be used to replicate a 'free-living' environment (chapter three). A third aim was to establish a robust method to assess 'free-living' energy intake (chapter four), since in the three paediatric studies available only Dodd, Welsman and Armstrong (2008) explored free-living energy intake, however there was no assessment of how accurate their energy intake measurement was.

Chapter three of this thesis provided an important foundation for subsequent studies in the present work (chapters five and six). Accurate determination of exercise intensity in exercise and appetite studies is imperative as it enables energy intake and appetite data to be interpreted appropriately. Consequently, the modified netball fitness test developed in this thesis was demonstrated to be a more meaningful test for assessing netball-specific aerobic power and endurance capability of adolescent female netball players, due to its ability to detect sport-specific endurance capabilities of the netball players, in comparison to an incremental treadmill test and the multi-stage fitness test. Thus the exercise intensity values for the netball exercise bouts identified in chapters five and six were determined using the modified netball fitness test developed in chapter three of this thesis, in order to assess netball-specific aerobic power as accurately as possible. It is therefore advantageous to employ $\dot{V}O_{2\,peak}$ protocols and FLEX heart rate techniques which include physical activity patterns that are representative of free-living habits in the sample population. Consequently, the development of the netball fitness test enabled more meaningful predictions of exercise intensity to be made whilst the girls were completing the netball exercise intervention in chapters five and six. In addition, the activity patterns established in the netball fitness test were used during the preliminary FLEX heart rate assessment and netball intervention (chapters five and six) and therefore enabled exercise-induced energy expenditure and 24-hour energy expenditure in chapters five and six to be quantified more appropriately. Previous exercise and appetite studies involving paediatric groups (Moore *et al*., 2004; Dodd, Welsman & Armstrong, 2008) have used laboratory based incremental cycling during FLEX heart rate and $\dot{V}O_{2\ peak}$ assessments and then ergometer based cycling for the subsequent exercise intervention. However, such exercise is not representative of young people's activity patterns, therefore a recommendation from chapter three is that FLEX heart rate methods, $\dot{V}O_{2\ peak}$ assessments and subsequent exercise interventions, in exercise and appetite research studies should replicate the free-living activity patterns of the sample population as closely as possible.

Chapter four of the present thesis also provided a basis for the research completed in chapters five and six. With regards to the measurement of energy intake in the three short-term paediatric exercise and appetite intervention studies to date, two monitored laboratory based food intake following prescribed exercise using laboratory-based *ad libitum* food weighing and observation (Moore *et al.*, 2004; Rumbold & Dodd, 2007) and one explored free-living energy intake (Dodd, Welsman & Armstrong, 2008). Laboratory-based *ad libitum* food weighing and observation, are not realistic methods for use in a free-living environment as they require researchers to prepare, record and weigh food and fluid items. Laboratory conditions are unrealistic environments for young people to be in. Therefore, exercise and appetite research studies should attempt to replicate environments (free-living) which are representative of the sample population, whereby typical food intake behaviours following exercise can be explored. However, in order to do this it is imperative that robust methods are developed to investigate energy intake in a free-living environment. Studies which have explored free-living energy intake have been adult based and have typically used self-reported, weighed food diaries (Stubbs *et al*., 2002b), without an indication of how accurate the energy intake assessment method was. Indeed, in adolescent girls the importance of assessing the accuracy of energy intake reporting is emphasised, given that this population typically under-report energy intake by 20-30% (Bandini *et al*., 1990; Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003). Subsequently, it is vital that the accuracy of energy intake assessment methods is explored to ensure robust data collection in exercise and appetite research studies.

Chapter four therefore explored the accuracy of a combined self-reported, weighed food diary and 24-hour recall interview technique for assessment of energy intake in a group (n=13) of trained adolescent (14-16 years) netball players over 24-hours. A slight overestimation of energy intake (4.2%) was reported, which was partially due to the notion of 'phantom foods' (as termed by Meredith *et al*., 1951), in combination with secondary food and drink items (confectionary and soft drinks) being over-reported and left over food and drink items not being weighed after consumption. Interestingly, a similar finding with regards to an over-reporting of energy intake has recently been identified in a preliminary study as part of the most recent National Diet and Nutrition Survey (Stephen *et al*., 2009). The study compared energy intake assessed using a 4-day estimated food diary and energy expenditure assessed using the doubly labelled water technique. The results found that young girls aged 4-10 years over-reported their energy intake, compared with the estimates of energy expenditure, which is in line with the over-reporting identified in chapter four of this thesis. On a group basis however the combined energy intake assessment method in chapter four indicated acceptable agreement, identified by the 95% confidence interval for bias ranging from 0.00 to 0.92 $MJ\cdot d^{-1}$. The agreement for the combined energy intake assessment technique was demonstrated to be an ‘acceptable’ technique to use and therefore was subsequently employed in chapters five and six to assess free-living energy intake. A recommendation based on the findings from chapter four suggests that an energy intake assessment tool which provides a 95% confidence interval of between 0.00 to 0.92 $MJ\cdot d^{-1}$, can be deemed ‘acceptable’ and therefore suitable for use on a group basis. Conversely to the over-reporting finding, under-reporting has typically been identified in adolescent females (Livingstone *et al*., 1992b; Bratteby *et al*., 1998; Bandini *et al*., 2003), therefore this finding was unexpected. Indeed, this finding clearly demonstrates the need to establish and evaluate the bias of energy intake assessment methods for given populations that researchers are working with. This is particularly important when a variable such as exercise is being manipulated to then explore subsequent alterations in appetite or food intake. Therefore, a recommendation from chapter four of this thesis is that all studies which aim to explore energy intake responses to a manipulated variable (food or drink preload, exercise intervention etc), should firstly establish the accuracy of the energy intake assessment method being used, specific to the sample population.

The establishment of the methods explored in chapters three and four ensured that robust data could be collected in the subsequent two short-term intervention studies (chapters five and six) and that the data was ecologically valid and as accurate as possible. The completion of these two studies enabled the main aim of the thesis to be investigated, which was to explore free-living energy intake and appetite (subjective and objective) following sport-specific exercise in adolescent girls.

A unique finding was identified in chapter five of this thesis, in which the trained (n=11) adolescent girls (13-15 years) undergoing representative intermittent netball exercise, increased their energy intake over a 2-day (48-hour) period when compared to a sedentary intervention. In one of the original cross-sectional studies which monitored habitual physical activity and free-living energy intake of lean, trained, male cadets over 14 days, a similar two day 'lag' in increased energy intake has been previously demonstrated (Edholm *et al*., 1955). The similar findings of the present work suggest that sport-specific, or at least exercise representative of the sample population's normal daily activities, might be key to explaining this partial compensation. The girls in chapter five of the present thesis also reported that they felt significantly hungrier immediately following the 40 minute netball exercise bout compared to immediately before. A similar elevation in hunger following cycling exercise at 75% $\dot{V}O_{2\ peak}$ has been identified in 10-11 year old overweight girls (Dodd, Welsman & Armstrong, 2008). Similar to adults, the effect of exercise on subjective appetite ratings in paediatric groups does not appear to be prolonged, but where a brief suppression in appetite has been identified in adult males (King & Blundell, 1995), conversely in young girls increases in hunger and appetite (Dodd, Welsman & Armstrong, 2008) following exercise have been identified. The subjective nature of the VAS questions might be an important factor with regards to assessing subjective appetite in paediatric studies. However, there does seem to be some consistency here between studies and therefore a rationale for further exploration using an objective measure of hunger such as appetite hormone analysis.

The findings arising from chapter five were therefore further investigated in chapter six of this thesis. Appetite differences have been identified in paediatric populations more specifically between 10-11 year old lean and overweight girls (Dodd, Armstrong & Welsman, 2008). In addition chapter five identified that trained adolescent girls felt hungrier following netball exercise compared to immediately before. Although only in adult populations regular exercise has been seen to influence appetite sensations (King *et al*., 1999; Long, Hart & Morgan, 2002; Martins, Truby & Morgan, 2007), whilst it was also postulated that training for specific sports can influence total ghrelin levels (an objective indicator of hunger) (Christ *et al*., 2006; Jurimae *et al*., 2007; Juriame, Juriame & Purge, 2007; Vestergaard *et al*., 2007). Therefore, due to the elevation in hunger in chapter five, it was decided that trained netballers would be targeted, as per the previous study of this thesis, versus a group of matched girls, not regularly taking part in structured netball exercise during the week. At the time of writing, non-acylated ghrelin concentrations have been explored in adolescent swimmers (Jürimäe *et al*., 2007a), however no studies have objectively assessed appetite in the form of acylated ghrelin concentrations following exercise in children or adolescent populations. In addition, no paediatric exercise and appetite studies have used a sensitive measure of energy expenditure in the form of simultaneous heart rate and accelerometry, therefore this method was also introduced for the final study of this thesis. Consequently, chapter six set out to determine any differences between trained and untrained adolescent girls with regards to energy intake, subjective appetite and objective appetite following exercise, using more sensitive methods of appetite assessment and energy expenditure quantification.

The relatively small sample size used in chapter six of this thesis is acknowledged, however findings were explored for each group (trained, n=5; untrained, n=5) and then as a group as a whole. Subsequently, the final study of this thesis (chapter six) identified that untrained 13-15 year old girls (n=5) undergoing intermittent netball exercise, increased their free-living energy intake 3-days (72-hours) following a bout of netball exercise when compared to a sedentary condition. In addition, 20 minutes into the netball exercise, the trained (n=5) and untrained (n=5) girls reported that they felt significantly more full compared to the corresponding time in a sedentary condition. Interestingly, the findings in chapters five and six were not consistent, with chapter five identifying an elevation in energy intake over 2-days (48-hours) and elevated feelings of hunger immediately following the netball exercise compared to before, in a group of trained adolescent girls. Whilst chapter six identified an elevation in energy intake 3-days (72-hours) following a bout of netball exercise in a group of untrained adolescent girls and an elevation in fullness 20 minutes during the netball exercise in both trained and untrained adolescent girls. Despite the methodologies being extremely similar (lean female participants, same duration and type (netball) of exercise imposed, free-living environment), there is certainly some inconsistency in findings from chapters five and six. Therefore it is difficult to make specific recommendations with regards to when energy balance is restored in young athletes. It is however clear that full energy intake compensation for a single bout of exercise is not immediate. However, partial energy intake compensation is apparent over 2-days following a bout of netball exercise, which results in trained adolescent girls being in less of a negative energy balance compared to a sedentary condition (although not significant). It is difficult to relate these findings to other paediatric exercise and appetite research studies (Moore *et al.*, 2004; Rumbold & Dodd, 2007; Dodd, Welsman & Armstrong, 2008) since they did not explore 24-hour energy expenditure, therefore energy balance could not be established. It is recommended that future intervention studies of this nature should quantify 24-hour energy expenditure in order to explore 24-hour energy balance.

Considering the findings from chapters five and six as a whole, several conclusions can be drawn. In some population groups (trained adolescent girls), netball exercise can increase feelings of hunger and elevate food intake over the 48-hour period following the bout, although full energy intake compensation for the exercise-induced energy expenditure does not occur. Further exploration is required to establish whether performing exercise habitually or being trained in a particular sporting discipline maybe a factor which influences the ability of adolescent girls to regulate their appetite. Indeed, it has been suggested that certain individuals are able to compensate for an exercise-induced energy deficit. Whybrow et al. (2008) identified large individual adult variations in the extent of energy intake compensation following exercise and termed those who do, 'compensators' and those who do not, 'non-compensators'. Consequently, discrepancies between individuals who compensate and those who do not are yet to be identified. It may be the case that, where feasible, future exercise and appetite research studies analyse and interpret data on an individual basis in an attempt to identify characteristics which make some participants 'compensators' and others 'non-compensators'. In addition, the inconsistency in findings between chapters five and six, may have been due to the low sample size, having only five trained and five untrained participants in chapter six. The small sample was due to the acylated ghrelin assay and therefore cannulation procedure being introduced in chapter six in combination with a complex design which made the study logistically demanding, particularly when using adolescent participants.

7.1 Future Research Recommendations

The four studies conducted for this thesis, provide a baseline for further exercise and appetite research using trained and untrained adolescent populations. This was especially the case for chapters three and four having developed ecologically valid methods for the 'free-living' assessment of sport-specific aerobic power and energy intake, for use in the two remaining appetite and exercise intervention studies (chapters five and six). These important methods developed in chapters three and four were used to robustly explore energy intake and subjective appetite following sport-specific exercise in chapters five and six. Chapter six also explored acylated ghrelin concentrations following sport-specific exercise to provide an objective measure of hunger. To our knowledge this was the first study to explore the active form of ghrelin (acylated ghrelin) in an adolescent population, within the context of an exercise and appetite intervention study.

Following completion of the four studies included in this thesis, three main recommendations can be made. Firstly, it is vital that FLEX heart rate methods, $\dot{V}O_{2\,peak}$ assessments and subsequent exercise interventions, in exercise and appetite research studies replicate the free-living activity patterns of the sample population involved in the study. Consequently, exercise intensity, exercise-induced energy expenditure and daily energy expenditure can be predicted more accurately. Secondly, all exercise and appetite studies should aim to establish the accuracy of the energy intake assessment method being used, again specific to the sample population. Especially in free-living environments involving adolescent populations, this measure will ensure that energy intake is assessed as accurately as possible. A final recommendation based on the final two short-term intervention studies is that where feasible, future exercise and appetite research studies should quantify 24-hour energy expenditure. Therefore energy intake and energy expenditure data can be analyse and interpret on an individual basis to better establish when and if energy balance is restored in adolescent populations.

With regards to energy intake and subjective appetite following a single bout of sport-specific exercise in trained adolescent girls, the present thesis has provided a baseline for further work, which could potentially be pursued along various avenues. For example, future studies might impose several consecutive bouts of sport-specific exercise, similar to a regular week of training for the given population group and continue to monitor energy intake and appetite following such representative exercise. It would also be valuable to identify whether trained adolescent boys respond in a similar or different manner to trained adolescent girls, in terms of energy intake and appetite following sport-specific exercise. Certainly findings from adult studies provide a firm justification for such research to be conducted (King, Burley & Blundell, 1994; King *et al*., 1996).

Since findings from chapters five and six of the present thesis were not consistent, further studies directly comparing energy intake and appetite following exercise in trained and untrained adolescent girls need to be conducted, with an increase in the sample size of the groups (n>5). This will help to identify discrepancies between individuals who compensate and those who do not. This will hopefully provide some clarification of any inherent similarities or differences between these special populations. Indeed, this would also warrant investigation in trained and untrained adolescent boys. In addition, further research should explore plasma acylated ghrelin concentrations following different modes (for example, intermittent versus continuous, different sports), durations and intensities of exercise in adolescent populations, using a sufficient number of participants. It would also be of interest to investigate daily plasma acylated ghrelin concentrations in trained adolescent involved in team sports such as netball and compare these levels to their untrained peers. Importantly, the final study of this thesis was the first to explore acylated ghrelin concentrations in adolescents and therefore it is envisaged that this sets the standard for future exercise and appetite studies in an adolescent population.

APPENDIX A

Example of a consent form (school and participant) and information sheets (ethics documents)

Penny Rumbold
Post-Graduate Researcher
School of Psychology and Sport Sciences
Northumbria Univeristy
226 Northumberland Building
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NE1 8ST
Office Tel: +44(0)191 2437018
Mobile Contact: 0774 5703493
Email: penny.rumbold@unn.ac.uk

School Consent form for Heaton Manor School

PhD Research Project:
Energy intake and appetite responses to a single bout of sport-specific physical activity in adolescent girls.

I have read and understood all the information provided and I hereby **give / do not give *** consent for the above study to take place at the above named school.

***(please delete as applicable)**

Name:____
(please print)

Title:____
(please print)

Signed:______________________________________ Date:____________

The PhD supervisor would be delighted to discuss this project with you if there are any queries. Details as follows:

Dr Caroline Dodd
Senior Lecturer in Sport and Exercise Physiology
Programme leader MSc Nutrition and Psychological Sciences
School of Psychology and Sport Sciences
Northumbria University
Northumberland Building
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SCHOOL OF PSYCHOLOGY & SPORT SCIENCES
CONSENT FORM

Please complete section 1 & 2 prior to the start of the study.

Section 1 (parent/guardian)

I agree to my child……………………………………………………………………participating in a research project investigating how aerobic fitness values from a netball fitness test compare to a standardised field based test and a laboratory based fitness test. The exact details have been explained to me in the information sheet.

I have been given the opportunity to ask any questions and discuss the procedures the study entails with the organiser of the investigation. I understand that my child will have her height, weight, body composition, aerobic fitness and heart rate assessed.

I understand that my child will be required on four different occasions, one at Northumbria University City Campus, for approximately two hours and the remaining three at Heaton Manor School for approximately an hour and a half.

Your child may experience fatigue during and after all three fitness tests relevant to their own fitness level. They will not be asked or expected to exercise beyond their own comfort level and if pain or discomfort is experienced it is important that they declare this immediately. Psychologically no discomfort is expected.

Importantly by agreeing for your child to take part you will be significantly helping to investigate and increase knowledge of an area greatly lacking in research.

The results will be saved on a computer in coded form and will therefore be completely confidential. Your child is free to withdraw at any time and does not have to provide a reason. It is emphasised that her relationship with the researcher and Junior club will be unaffected. The results of this project may be published, but the information will not be linked to any specific person. You will be provided with a copy of your child's results if you wish to have them. You can ask questions about the study at any time. If your child has any medical conditions, which affect their participation in exercise they are advised not to take part. Please do not hesitate to contact either myself using the details provided on the covering letter, or Dr. Caroline Dodd (the project supervisor: 0191 2437553), to discuss any queries further.

Signed………………………………(parent/guardian) **Signed ……………………(researcher)**

Name (Printed)………………………… **Name (Printed)…………………………**

Date…………………… **Date………………………..**

In addition, transport will be provided, if you (parent/guardian) agrees to the researcher (myself, Penny Rumbold) driving your child to and from Northumbria University / school.

Signed …………………………………………………(parent/guardian) Date ……………………..

Section 2 (child)

I,……………………………………………………….agree to taking part in the study described above, which has been explained to me. I understand what is needed of me during the study and that I can withdraw from the study at any time.

Signed…………………………………………………..(child) **Date …………………...**

PLEASE RETURN IN THE SELF-ADDRESSED ENVELOPE PROVIDED

Post-Graduate Researcher
School of Psychology and Sport Sciences
Northumbria Univeristy
226 Northumberland Building
Newcastle upon Tyne
NE1 8ST
Office Tel: +44(0)191 2437018
Mobile Contact: 0774 5703493
Email: penny.rumbold@unn.ac.uk

MEDICAL QUESTIONNAIRE

- **Name of child:**____________________ **Date of Birth:**__________________
- Does your daughter suffer from any physical/medical conditions, which could be affected by exercise? YES / NO. If YES, please give details:

____
____

- Does your daughter normally carry any medication? YES / NO. If YES, please give details:

____
____

- To the best of your knowledge has your daughter been in contact with any contagious or infectious diseases or suffered from anything in the last four weeks, which could become contagious or infectious? YES / NO. If YES, please give details:

____

- Has your daughter started her periods yet? (This information is required to ensure a similar maturation status in our participant group). YES / NO

- Does your child have any special dietary requirements or food allergies? YES / NO. If YES, please give details:

____
____
____

- Any further details regarding your child's medical status which we should be aware of:

____
____

- I may be contacted by telephoning (please include STD code):

Home _________________ Work ________________ Mobile _________________
Address ____

- If I am not available, please contact: ______________________________________
Telephone number (including STD code): _________________________________
- Name, address and telephone number of family doctor:

____
____

Signed ____________________Parent/Guardian Date _____________

Printed ____________________Parent/Guardian

PLEASE RETURN IN THE SELF-ADDRESSED ENVELOPE PROVIDED

Penny Rumbold
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Information for Parents

Thank you for taking the time to read this information concerning the study in which your child has been asked to participate. I am interested in finding out how aerobic fitness values from a netball specific fitness test compare to a standardised field based fitness test (bleep test) and a laboratory based fitness test (treadmill test) in adolescent girls. The School of Psychology and Sport Science's Ethics Committee, at Northumbria University have approved the study and all the procedures involved. Please read all the information carefully in order to decide whether you agree to your child being involved. If you decide to allow your child to participate you will be required to sign the form of consent and medical questionnaire. Your child will also be required to sign the consent form after reading the information sheet provided. If you decide that you do not want your child to be involved there will be no disadvantage to them and neither yours nor your child's relationship with either the school or the university will be affected.

Project Title – Assessing peak oxygen uptake in active adolescent girls age 13-15 years, using a netball fitness test.

Procedure Breakdown

One preliminary visit to Northumbria University will take place (visit 1). The protocol will then involve three more test days (visits 2, 3 and 4), which will take place at Heaton Manor School straight after your daughter has finished lessons. These visits will be separated by one week. Please note the girls will be in a group of three and therefore will not be alone on testing dates. Transport will be provided by myself (Penny Rumbold) to and from Northumbria University / Heaton Manor School when required.

VISIT 1 **Body composition** Northumbria University Your daughter will be picked up from Heaton Manor School ON: ________________________ AT: approx. 15:00pm after lessons And will be returned home for approximately 17:00pm.	***Body Composition*:** **Height and weight will be assessed along with body composition, using the Bod Pod. Knowledge from previous research indicates that children find this very enjoyable. All body composition tests are non-invasive and will be conducted in a private room by myself (Penny Rumbold) or and another female researcher.** **Bod Pod – The Bod Pod uses the latest technology to measure how much of the body is fat tissue and how much is lean tissue. Wearing a** swimming costume**, the child simply sits inside the egg-shaped Bod Pod capsule. The measurement process takes about two minutes.**
VISIT 2, 3 and 4 VISIT 2 Heaton Manor School ON: ________________________ AT: approx. 15:00pm after lessons. Will be returned home for 16:30pm. VISIT 3 Heaton Manor School ON: ________________________ AT: approx. 15:00pm after lessons.	Your child will perform each of these tests, in a random order. One test will be completed on visit 2, visit 3 and visit 4. **Your daughter will need sports kit on visit 2, 3 and 4.** Prior to each test your daughter must: **1. Refrain from eating 2 hours before.** **2. Avoid any exercise on the test day.** ***Netball Fitness Test:*** This standard fitness test lasts approximately 22 minutes, in which the exercise intensity is raised progressively from very easy to very hard (maximal), whilst completing netball based activities. Measurements of oxygen uptake, using a facemask and heart rate, using a watch-like piece of equipment, are taken at different intensities throughout. The test comprises of 11 levels, at the end of each level your daughter will be asked how easy or hard they

Will be returned home for 16:30pm. <u>VISIT 4</u> Heaton Manor School ON: _________________________ AT: approx. 15:00pm after lessons. Will be returned home for 16:30pm.	are finding the exercise. Your daughter will be encouraged to keep running through the test for as long as they can. ***Treadmill Running Test:*** This standard fitness test lasts approximately 20 minutes, where the exercise intensity is raised progressively from very easy to very hard (maximal), whilst running at various speeds and gradients on a treadmill. After the speed or gradient has been altered your daughter will be asked how easy or hard they are finding the exercise. Measurements of oxygen uptake, using a facemask and heart rate, using a watch-like piece of equipment, are taken at different intensities throughout. Your daughter will be encouraged to keep running through the test for as long as they can. ***Multi-Stage Fitness Test (Bleep Test):*** This standard fitness test lasts approximately 23 minutes depending on the level of exercise intensity achieved. It comprises of 23 levels, at the end of each level your daughter will be asked how easy or hard they are finding the exercise. Heart rate measurements will be collected using a watch-like piece of equipment throughout the test.

<u>Safety</u>
Fully qualified technical staff will be present when required and qualified first aid personnel will be in attendance at all times.

<u>Can participation be ceased?</u>
You and your daughter can change your mind at any time and decide not to continue participating. If withdrawal does occur reasons for this do not have to be declared and no disadvantages will be experienced.

Thank you for taking the time to read this information.

Penny Rumbold

Penny Rumbold
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Congratulations!

You are being asked to take part in this exciting study because you're an excellent netball player. This means that you will be invited to visit a university laboratory to perform some fun tests and then on three other occasions you get to do some exercise. If that sounds good just read on and see if you still like the idea at the end!

Now for the exciting part………………………………

Visit 1

One night after school you will be invited into the University laboratory. Here you will get to wear your swimming costume and sit in a huge egg so that other types of measurements can be taken. It is very important that before this test you don't eat or exercise for 2 hours before.

Visit 2, 3 and 4

You and 2 other friends will perform 3 different tests, which are described below. On visit 2 one test will be completed, on visit 3 another test will be completed and on visit 4 the last test will be completed.

Before these tests you will have to follow two rules:

1. **Avoid eating for 2 hours prior to the test.**
2. **Avoid exercising on the day of the test.**

Netball Specific Exercise Test

This test will take approximately 22 minutes. You will be asked to complete some netball activities, which will be very easy at the beginning and then will get harder towards the end. You will be asked to wear a heart rate strap and a face mask to measure how much oxygen you are using for the exercise. When you are exercising it has to be to your best effort so we can see how fit you are.

Treadmill Running Test

This test will take approximately 20 minutes. You will be asked to run on a treadmill at different speeds and gradients, which will be very easy at the beginning and then will get harder towards the end. You will be asked to wear a heart rate strap and a face mask to measure how much oxygen you are using for the exercise. When you are exercising it has to be to your best effort so we can see how fit you are.

Multi-Stage Fitness Test

This test will take approximately 23 minutes. You will be asked to complete shuttle runs, which will be very easy at the beginning and then will get harder towards the end. You will be asked to wear a heart rate strap whilst you are exercising. When you are exercising it has to be to your best effort so we can see how fit you are.

So……………………………………

If all of this sounds good and you want to take part then that's great, just let your parents know. If you can't take part or you don't want to that is fine too, but remember you are still a good player. If you start the study and you don't like it and want to drop out then that is no problem either.

Thank you, for reading this! Hope you are as excited as I am!

Penny

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Assessing peak oxygen uptake in active adolescent girls age 13-15 years, using a netball fitness test.

Participant (Childs) Debrief

Participant Number ___________

This study explored how fitness values from a netball fitness test compare to a standardised field based fitness test (bleep test) and a laboratory based fitness test (treadmill test) in adolescent girls. You were asked to complete two field based tests, known as the bleep test and a netball specific fitness test and also one laboratory based running test on a treadmill. Whilst you were doing these tests your oxygen and heart rate levels were measured so that they could be compared between the three different tests.

No individual data will be available but a summarised form will be available. If you would like to receive the summary or if you have any questions regarding the experiment please contact Penny Rumbold (email: penny.rumbold@unn.ac.uk phone: **0191 243 7018**). You are also reminded of your right to withdraw from the study at any time. If you choose to do so, please use the email address above, giving your confidential participant number code (on top of this sheet) and all your data will be deleted.

If you have any concerns or worries concerning the way in which this research has been conducted, or if you have requested but do not receive feedback from the principal investigator concerning the general outcomes of the study within a few weeks after the study is concluded, then please contact Professor Kenny Coventry (Associate Dean Research) via email at kenny.coventry@unn.ac.uk, or via telephone on 0191 243 7027.

Thank you for your participation.

Penny Rumbold

APPENDIX B

ANOVA outputs

CHAPTER 3

Peak Oxygen Uptake

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
test	Sphericity Assumed	128.822	2	64.411	8.947	.001
	Greenhouse-Geisser	128.822	1.394	92.385	8.947	.005
	Huynh-Feldt	128.822	1.534	83.990	8.947	.004
	Lower-bound	128.822	1.000	128.822	8.947	.012
Error(test)	Sphericity Assumed	158.385	22	7.199		
	Greenhouse-Geisser	158.385	15.338	10.326		
	Huynh-Feldt	158.385	16.872	9.388		
	Lower-bound	158.385	11.000	14.399		

Heart Rate at $\dot{V}O_{2\ peak}$

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
test	Sphericity Assumed	260.167	2	130.083	3.394	.052
	Greenhouse-Geisser	260.167	1.992	130.633	3.394	.052
	Huynh-Feldt	260.167	2.000	130.083	3.394	.052
	Lower-bound	260.167	1.000	260.167	3.394	.093
Error(test)	Sphericity Assumed	843.167	22	38.326		
	Greenhouse-Geisser	843.167	21.907	38.488		
	Huynh-Feldt	843.167	22.000	38.326		
	Lower-bound	843.167	11.000	76.652		

Respiratory Exchange Ratio at $\dot{V}O_{2\ peak}$

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
test	Sphericity Assumed	.024	1	.024	3.595	.085
	Greenhouse-Geisser	.024	1.000	.024	3.595	.085
	Huynh-Feldt	.024	1.000	.024	3.595	.085
	Lower-bound	.024	1.000	.024	3.595	.085
Error(test)	Sphericity Assumed	.074	11	.007		
	Greenhouse-Geisser	.074	11.000	.007		
	Huynh-Feldt	.074	11.000	.007		
	Lower-bound	.074	11.000	.007		

Ratings of Perceived Exertion

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
test	Sphericity Assumed	13.167	2	6.583	6.952	.005
	Greenhouse-Geisser	13.167	1.539	8.556	6.952	.010
	Huynh-Feldt	13.167	1.741	7.565	6.952	.007
	Lower-bound	13.167	1.000	13.167	6.952	.023
Error(test)	Sphericity Assumed	20.833	22	.947		
	Greenhouse-Geisser	20.833	16.928	1.231		
	Huynh-Feldt	20.833	19.146	1.088		
	Lower-bound	20.833	11.000	1.894		

Time to Reach $\dot{V}O_2$ peak

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
tests	Sphericity Assumed	891.211	2	445.605	136.767	.000
	Greenhouse-Geisser	891.211	1.523	585.082	136.767	.000
	Huynh-Feldt	891.211	1.718	518.826	136.767	.000
	Lower-bound	891.211	1.000	891.211	136.767	.000
Error(tests)	Sphericity Assumed	71.679	22	3.258		
	Greenhouse-Geisser	71.679	16.755	4.278		
	Huynh-Feldt	71.679	18.895	3.794		
	Lower-bound	71.679	11.000	6.516		

Energy Expenditure:

Days 1 and 2 (Maintenance Days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.118	1	.118	.108	.750
	Greenhouse-Geisser	.118	1.000	.118	.108	.750
	Huynh-Feldt	.118	1.000	.118	.108	.750
	Lower-bound	.118	1.000	.118	.108	.750
Error(cond)	Sphericity Assumed	10.976	10	1.098		
	Greenhouse-Geisser	10.976	10.000	1.098		
	Huynh-Feldt	10.976	10.000	1.098		
	Lower-bound	10.976	10.000	1.098		
day	Sphericity Assumed	.114	1	.114	.127	.728
	Greenhouse-Geisser	.114	1.000	.114	.127	.728
	Huynh-Feldt	.114	1.000	.114	.127	.728
	Lower-bound	.114	1.000	.114	.127	.728
Error(day)	Sphericity Assumed	8.945	10	.894		
	Greenhouse-Geisser	8.945	10.000	.894		
	Huynh-Feldt	8.945	10.000	.894		
	Lower-bound	8.945	10.000	.894		
cond * day	Sphericity Assumed	1.008	1	1.008	.966	.349
	Greenhouse-Geisser	1.008	1.000	1.008	.966	.349
	Huynh-Feldt	1.008	1.000	1.008	.966	.349
	Lower-bound	1.008	1.000	1.008	.966	.349
Error(cond*day)	Sphericity Assumed	10.438	10	1.044		
	Greenhouse-Geisser	10.438	10.000	1.044		
	Huynh-Feldt	10.438	10.000	1.044		
	Lower-bound	10.438	10.000	1.044		

Day 3 NSEP and SED Interventions

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	6.866	1	6.866	385.258	.000000
	Greenhouse-Geisser	6.866	1.000	6.866	385.258	.000
	Huynh-Feldt	6.866	1.000	6.866	385.258	.000
	Lower-bound	6.866	1.000	6.866	385.258	.000
Error(cond)	Sphericity Assumed	.178	10	.018		
	Greenhouse-Geisser	.178	10.000	.018		
	Huynh-Feldt	.178	10.000	.018		
	Lower-bound	.178	10.000	.018		

Ratings of Perceived Exertion during the NSEP

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	191.818	4	47.955	88.063	.000
	Greenhouse-Geisser	191.818	1.467	130.732	88.063	.000
	Huynh-Feldt	191.818	1.657	115.753	88.063	.000
	Lower-bound	191.818	1.000	191.818	88.063	.000
Error(time)	Sphericity Assumed	21.782	40	.545		
	Greenhouse-Geisser	21.782	14.673	1.485		
	Huynh-Feldt	21.782	16.571	1.314		
	Lower-bound	21.782	10.000	2.178		

Heart Rate during the NSEP

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	45667.527	4	11416.882	142.441	.000
	Greenhouse-Geisser	45667.527	1.754	26043.448	142.441	.000
	Huynh-Feldt	45667.527	2.096	21782.891	142.441	.000
	Lower-bound	45667.527	1.000	45667.527	142.441	.000
Error(time)	Sphericity Assumed	3206.073	40	80.152		
	Greenhouse-Geisser	3206.073	17.535	182.837		
	Huynh-Feldt	3206.073	20.965	152.926		
	Lower-bound	3206.073	10.000	320.607		

Days 3, 4 and 5 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	2.919	1	2.919	1.149	.309
	Greenhouse-Geisser	2.919	1.000	2.919	1.149	.309
	Huynh-Feldt	2.919	1.000	2.919	1.149	.309
	Lower-bound	2.919	1.000	2.919	1.149	.309
Error(cond)	Sphericity Assumed	25.402	10	2.540		
	Greenhouse-Geisser	25.402	10.000	2.540		
	Huynh-Feldt	25.402	10.000	2.540		
	Lower-bound	25.402	10.000	2.540		
day	Sphericity Assumed	7.005	2	3.502	4.244	.029
	Greenhouse-Geisser	7.005	1.719	4.075	4.244	.037
	Huynh-Feldt	7.005	2.000	3.502	4.244	.029
	Lower-bound	7.005	1.000	7.005	4.244	.066
Error(day)	Sphericity Assumed	16.505	20	.825		
	Greenhouse-Geisser	16.505	17.190	.960		
	Huynh-Feldt	16.505	20.000	.825		
	Lower-bound	16.505	10.000	1.650		
cond * day	Sphericity Assumed	13.297	2	6.649	6.356	.007
	Greenhouse-Geisser	13.297	1.376	9.665	6.356	.018
	Huynh-Feldt	13.297	1.523	8.732	6.356	.014
	Lower-bound	13.297	1.000	13.297	6.356	.030
Error(cond*day)	Sphericity Assumed	20.920	20	1.046		
	Greenhouse-Geisser	20.920	13.758	1.521		
	Huynh-Feldt	20.920	15.228	1.374		
	Lower-bound	20.920	10.000	2.092		

Day 3 Energy Expenditure NSEP versus SED

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	15.406	1	15.406	16.229	.002
	Greenhouse-Geisser	15.406	1.000	15.406	16.229	.002
	Huynh-Feldt	15.406	1.000	15.406	16.229	.002
	Lower-bound	15.406	1.000	15.406	16.229	.002
Error(cond)	Sphericity Assumed	9.493	10	.949		
	Greenhouse-Geisser	9.493	10.000	.949		
	Huynh-Feldt	9.493	10.000	.949		
	Lower-bound	9.493	10.000	.949		

Energy Intake:

Days 1 and 2 (Maintenance days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.966	1	1.966	1.002	.340
	Greenhouse-Geisser	1.966	1.000	1.966	1.002	.340
	Huynh-Feldt	1.966	1.000	1.966	1.002	.340
	Lower-bound	1.966	1.000	1.966	1.002	.340
Error(cond)	Sphericity Assumed	19.616	10	1.962		
	Greenhouse-Geisser	19.616	10.000	1.962		
	Huynh-Feldt	19.616	10.000	1.962		
	Lower-bound	19.616	10.000	1.962		
day	Sphericity Assumed	8.378	1	8.378	1.856	.203
	Greenhouse-Geisser	8.378	1.000	8.378	1.856	.203
	Huynh-Feldt	8.378	1.000	8.378	1.856	.203
	Lower-bound	8.378	1.000	8.378	1.856	.203
Error(day)	Sphericity Assumed	45.149	10	4.515		
	Greenhouse-Geisser	45.149	10.000	4.515		
	Huynh-Feldt	45.149	10.000	4.515		
	Lower-bound	45.149	10.000	4.515		
cond * day	Sphericity Assumed	.042	1	.042	.072	.794
	Greenhouse-Geisser	.042	1.000	.042	.072	.794
	Huynh-Feldt	.042	1.000	.042	.072	.794
	Lower-bound	.042	1.000	.042	.072	.794
Error(cond*day)	Sphericity Assumed	5.832	10	.583		
	Greenhouse-Geisser	5.832	10.000	.583		
	Huynh-Feldt	5.832	10.000	.583		
	Lower-bound	5.832	10.000	.583		

Day 3 NSEP and SED Interventions

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
COND	Sphericity Assumed	40.909	1	40.909	.413	.535
	Greenhouse-Geisser	40.909	1.000	40.909	.413	.535
	Huynh-Feldt	40.909	1.000	40.909	.413	.535
	Lower-bound	40.909	1.000	40.909	.413	.535
Error(COND)	Sphericity Assumed	991.091	10	99.109		
	Greenhouse-Geisser	991.091	10.000	99.109		
	Huynh-Feldt	991.091	10.000	99.109		
	Lower-bound	991.091	10.000	99.109		

Days 3, 4 and 5 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	5.952	1	5.952	2.143	.174
	Greenhouse-Geisser	5.952	1.000	5.952	2.143	.174
	Huynh-Feldt	5.952	1.000	5.952	2.143	.174
	Lower-bound	5.952	1.000	5.952	2.143	.174
Error(cond)	Sphericity Assumed	27.777	10	2.778		
	Greenhouse-Geisser	27.777	10.000	2.778		
	Huynh-Feldt	27.777	10.000	2.778		
	Lower-bound	27.777	10.000	2.778		
day	Sphericity Assumed	18.134	2	9.067	2.289	.127
	Greenhouse-Geisser	18.134	1.711	10.596	2.289	.137
	Huynh-Feldt	18.134	2.000	9.067	2.289	.127
	Lower-bound	18.134	1.000	18.134	2.289	.161
Error(day)	Sphericity Assumed	79.234	20	3.962		
	Greenhouse-Geisser	79.234	17.114	4.630		
	Huynh-Feldt	79.234	20.000	3.962		
	Lower-bound	79.234	10.000	7.923		
cond * day	Sphericity Assumed	5.520	2	2.760	1.022	.378
	Greenhouse-Geisser	5.520	1.909	2.892	1.022	.375
	Huynh-Feldt	5.520	2.000	2.760	1.022	.378
	Lower-bound	5.520	1.000	5.520	1.022	.336
Error(cond*day)	Sphericity Assumed	54.001	20	2.700		
	Greenhouse-Geisser	54.001	19.088	2.829		
	Huynh-Feldt	54.001	20.000	2.700		
	Lower-bound	54.001	10.000	5.400		

48-hour Energy Intake

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	21.463	1	21.463	8.611	.015
	Greenhouse-Geisser	21.463	1.000	21.463	8.611	.015
	Huynh-Feldt	21.463	1.000	21.463	8.611	.015
	Lower-bound	21.463	1.000	21.463	8.611	.015
Error(cond)	Sphericity Assumed	24.925	10	2.493		
	Greenhouse-Geisser	24.925	10.000	2.493		
	Huynh-Feldt	24.925	10.000	2.493		
	Lower-bound	24.925	10.000	2.493		

Macronutrient Intake

Days 1 and 2 (Maintenance days)

Fat

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.451	1	1.451	.286	.604
	Greenhouse-Geisser	1.451	1.000	1.451	.286	.604
	Huynh-Feldt	1.451	1.000	1.451	.286	.604
	Lower-bound	1.451	1.000	1.451	.286	.604
Error(cond)	Sphericity Assumed	50.665	10	5.067		
	Greenhouse-Geisser	50.665	10.000	5.067		
	Huynh-Feldt	50.665	10.000	5.067		
	Lower-bound	50.665	10.000	5.067		
day	Sphericity Assumed	27.889	1	27.889	.727	.414
	Greenhouse-Geisser	27.889	1.000	27.889	.727	.414
	Huynh-Feldt	27.889	1.000	27.889	.727	.414
	Lower-bound	27.889	1.000	27.889	.727	.414
Error(day)	Sphericity Assumed	383.834	10	38.383		
	Greenhouse-Geisser	383.834	10.000	38.383		
	Huynh-Feldt	383.834	10.000	38.383		
	Lower-bound	383.834	10.000	38.383		
cond * day	Sphericity Assumed	1.302	1	1.302	.192	.671
	Greenhouse-Geisser	1.302	1.000	1.302	.192	.671
	Huynh-Feldt	1.302	1.000	1.302	.192	.671
	Lower-bound	1.302	1.000	1.302	.192	.671
Error(cond*day)	Sphericity Assumed	67.834	10	6.783		
	Greenhouse-Geisser	67.834	10.000	6.783		
	Huynh-Feldt	67.834	10.000	6.783		
	Lower-bound	67.834	10.000	6.783		

CHO

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.162	1	1.162	.127	.729
	Greenhouse-Geisser	1.162	1.000	1.162	.127	.729
	Huynh-Feldt	1.162	1.000	1.162	.127	.729
	Lower-bound	1.162	1.000	1.162	.127	.729
Error(cond)	Sphericity Assumed	91.833	10	9.183		
	Greenhouse-Geisser	91.833	10.000	9.183		
	Huynh-Feldt	91.833	10.000	9.183		
	Lower-bound	91.833	10.000	9.183		
day	Sphericity Assumed	127.602	1	127.602	3.077	.110
	Greenhouse-Geisser	127.602	1.000	127.602	3.077	.110
	Huynh-Feldt	127.602	1.000	127.602	3.077	.110
	Lower-bound	127.602	1.000	127.602	3.077	.110
Error(day)	Sphericity Assumed	414.651	10	41.465		
	Greenhouse-Geisser	414.651	10.000	41.465		
	Huynh-Feldt	414.651	10.000	41.465		
	Lower-bound	414.651	10.000	41.465		
cond * day	Sphericity Assumed	4.173	1	4.173	.302	.595
	Greenhouse-Geisser	4.173	1.000	4.173	.302	.595
	Huynh-Feldt	4.173	1.000	4.173	.302	.595
	Lower-bound	4.173	1.000	4.173	.302	.595
Error(cond*day)	Sphericity Assumed	138.235	10	13.823		
	Greenhouse-Geisser	138.235	10.000	13.823		
	Huynh-Feldt	138.235	10.000	13.823		
	Lower-bound	138.235	10.000	13.823		

Protein

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	3.912	1	3.912	.828	.384
	Greenhouse-Geisser	3.912	1.000	3.912	.828	.384
	Huynh-Feldt	3.912	1.000	3.912	.828	.384
	Lower-bound	3.912	1.000	3.912	.828	.384
Error(cond)	Sphericity Assumed	47.246	10	4.725		
	Greenhouse-Geisser	47.246	10.000	4.725		
	Huynh-Feldt	47.246	10.000	4.725		
	Lower-bound	47.246	10.000	4.725		
day	Sphericity Assumed	36.110	1	36.110	1.878	.201
	Greenhouse-Geisser	36.110	1.000	36.110	1.878	.201
	Huynh-Feldt	36.110	1.000	36.110	1.878	.201
	Lower-bound	36.110	1.000	36.110	1.878	.201
Error(day)	Sphericity Assumed	192.313	10	19.231		
	Greenhouse-Geisser	192.313	10.000	19.231		
	Huynh-Feldt	192.313	10.000	19.231		
	Lower-bound	192.313	10.000	19.231		
cond * day	Sphericity Assumed	.364	1	.364	.101	.757
	Greenhouse-Geisser	.364	1.000	.364	.101	.757
	Huynh-Feldt	.364	1.000	.364	.101	.757
	Lower-bound	.364	1.000	.364	.101	.757
Error(cond*day)	Sphericity Assumed	36.000	10	3.600		
	Greenhouse-Geisser	36.000	10.000	3.600		
	Huynh-Feldt	36.000	10.000	3.600		
	Lower-bound	36.000	10.000	3.600		

Days 3, 4 and 5 (Intervention day and follow-up days)

Fat

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.994	1	.994	.040	.845
	Greenhouse-Geisser	.994	1.000	.994	.040	.845
	Huynh-Feldt	.994	1.000	.994	.040	.845
	Lower-bound	.994	1.000	.994	.040	.845
Error(cond)	Sphericity Assumed	248.230	10	24.823		
	Greenhouse-Geisser	248.230	10.000	24.823		
	Huynh-Feldt	248.230	10.000	24.823		
	Lower-bound	248.230	10.000	24.823		
day	Sphericity Assumed	3.120	2	1.560	.093	.911
	Greenhouse-Geisser	3.120	1.770	1.763	.093	.890
	Huynh-Feldt	3.120	2.000	1.560	.093	.911
	Lower-bound	3.120	1.000	3.120	.093	.766
Error(day)	Sphericity Assumed	334.851	20	16.743		
	Greenhouse-Geisser	334.851	17.696	18.923		
	Huynh-Feldt	334.851	20.000	16.743		
	Lower-bound	334.851	10.000	33.485		
cond * day	Sphericity Assumed	50.309	2	25.155	2.350	.121
	Greenhouse-Geisser	50.309	1.865	26.974	2.350	.126
	Huynh-Feldt	50.309	2.000	25.155	2.350	.121
	Lower-bound	50.309	1.000	50.309	2.350	.156
Error(cond*day)	Sphericity Assumed	214.060	20	10.703		
	Greenhouse-Geisser	214.060	18.651	11.477		
	Huynh-Feldt	214.060	20.000	10.703		
	Lower-bound	214.060	10.000	21.406		

CHO

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	28.211	1	28.211	.880	.370
	Greenhouse-Geisser	28.211	1.000	28.211	.880	.370
	Huynh-Feldt	28.211	1.000	28.211	.880	.370
	Lower-bound	28.211	1.000	28.211	.880	.370
Error(cond)	Sphericity Assumed	320.416	10	32.042		
	Greenhouse-Geisser	320.416	10.000	32.042		
	Huynh-Feldt	320.416	10.000	32.042		
	Lower-bound	320.416	10.000	32.042		
day	Sphericity Assumed	47.585	2	23.792	.920	.415
	Greenhouse-Geisser	47.585	1.838	25.894	.920	.408
	Huynh-Feldt	47.585	2.000	23.792	.920	.415
	Lower-bound	47.585	1.000	47.585	.920	.360
Error(day)	Sphericity Assumed	516.960	20	25.848		
	Greenhouse-Geisser	516.960	18.377	28.131		
	Huynh-Feldt	516.960	20.000	25.848		
	Lower-bound	516.960	10.000	51.696		
cond * day	Sphericity Assumed	17.085	2	8.542	.422	.662
	Greenhouse-Geisser	17.085	1.584	10.783	.422	.617
	Huynh-Feldt	17.085	1.833	9.319	.422	.645
	Lower-bound	17.085	1.000	17.085	.422	.531
Error(cond*day)	Sphericity Assumed	405.176	20	20.259		
	Greenhouse-Geisser	405.176	15.844	25.573		
	Huynh-Feldt	405.176	18.333	22.101		
	Lower-bound	405.176	10.000	40.518		

Protein

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	20.754	1	20.754	1.535	.244
	Greenhouse-Geisser	20.754	1.000	20.754	1.535	.244
	Huynh-Feldt	20.754	1.000	20.754	1.535	.244
	Lower-bound	20.754	1.000	20.754	1.535	.244
Error(cond)	Sphericity Assumed	135.237	10	13.524		
	Greenhouse-Geisser	135.237	10.000	13.524		
	Huynh-Feldt	135.237	10.000	13.524		
	Lower-bound	135.237	10.000	13.524		
day	Sphericity Assumed	36.339	2	18.169	1.835	.186
	Greenhouse-Geisser	36.339	1.997	18.198	1.835	.186
	Huynh-Feldt	36.339	2.000	18.169	1.835	.186
	Lower-bound	36.339	1.000	36.339	1.835	.205
Error(day)	Sphericity Assumed	198.084	20	9.904		
	Greenhouse-Geisser	198.084	19.968	9.920		
	Huynh-Feldt	198.084	20.000	9.904		
	Lower-bound	198.084	10.000	19.808		
cond * day	Sphericity Assumed	10.246	2	5.123	.625	.545
	Greenhouse-Geisser	10.246	1.947	5.264	.625	.541
	Huynh-Feldt	10.246	2.000	5.123	.625	.545
	Lower-bound	10.246	1.000	10.246	.625	.447
Error(cond*day)	Sphericity Assumed	163.869	20	8.193		
	Greenhouse-Geisser	163.869	19.467	8.418		
	Huynh-Feldt	163.869	20.000	8.193		
	Lower-bound	163.869	10.000	16.387		

Energy Balance:

Days 1 and 2 (Maintenance days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.120	1	1.120	.255	.624
	Greenhouse-Geisser	1.120	1.000	1.120	.255	.624
	Huynh-Feldt	1.120	1.000	1.120	.255	.624
	Lower-bound	1.120	1.000	1.120	.255	.624
Error(cond)	Sphericity Assumed	43.870	10	4.387		
	Greenhouse-Geisser	43.870	10.000	4.387		
	Huynh-Feldt	43.870	10.000	4.387		
	Lower-bound	43.870	10.000	4.387		
day	Sphericity Assumed	10.447	1	10.447	2.370	.155
	Greenhouse-Geisser	10.447	1.000	10.447	2.370	.155
	Huynh-Feldt	10.447	1.000	10.447	2.370	.155
	Lower-bound	10.447	1.000	10.447	2.370	.155
Error(day)	Sphericity Assumed	44.075	10	4.408		
	Greenhouse-Geisser	44.075	10.000	4.408		
	Huynh-Feldt	44.075	10.000	4.408		
	Lower-bound	44.075	10.000	4.408		
cond * day	Sphericity Assumed	.638	1	.638	.376	.553
	Greenhouse-Geisser	.638	1.000	.638	.376	.553
	Huynh-Feldt	.638	1.000	.638	.376	.553
	Lower-bound	.638	1.000	.638	.376	.553
Error(cond*day)	Sphericity Assumed	16.972	10	1.697		
	Greenhouse-Geisser	16.972	10.000	1.697		
	Huynh-Feldt	16.972	10.000	1.697		
	Lower-bound	16.972	10.000	1.697		

Days 3, 4 and 5 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	2.095	1	2.095	.823	.386
	Greenhouse-Geisser	2.095	1.000	2.095	.823	.386
	Huynh-Feldt	2.095	1.000	2.095	.823	.386
	Lower-bound	2.095	1.000	2.095	.823	.386
Error(cond)	Sphericity Assumed	25.469	10	2.547		
	Greenhouse-Geisser	25.469	10.000	2.547		
	Huynh-Feldt	25.469	10.000	2.547		
	Lower-bound	25.469	10.000	2.547		
day	Sphericity Assumed	14.440	2	7.220	1.740	.201
	Greenhouse-Geisser	14.440	1.456	9.917	1.740	.211
	Huynh-Feldt	14.440	1.641	8.801	1.740	.208
	Lower-bound	14.440	1.000	14.440	1.740	.217
Error(day)	Sphericity Assumed	83.004	20	4.150		
	Greenhouse-Geisser	83.004	14.561	5.700		
	Huynh-Feldt	83.004	16.407	5.059		
	Lower-bound	83.004	10.000	8.300		
cond * day	Sphericity Assumed	17.382	2	8.691	2.702	.091
	Greenhouse-Geisser	17.382	1.944	8.941	2.702	.093
	Huynh-Feldt	17.382	2.000	8.691	2.702	.091
	Lower-bound	17.382	1.000	17.382	2.702	.131
Error(cond*day)	Sphericity Assumed	64.330	20	3.217		
	Greenhouse-Geisser	64.330	19.441	3.309		
	Huynh-Feldt	64.330	20.000	3.217		
	Lower-bound	64.330	10.000	6.433		

48-hour Energy Balance

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	2.577	1	2.577	.464	.511
	Greenhouse-Geisser	2.577	1.000	2.577	.464	.511
	Huynh-Feldt	2.577	1.000	2.577	.464	.511
	Lower-bound	2.577	1.000	2.577	.464	.511
Error(cond)	Sphericity Assumed	55.575	10	5.558		
	Greenhouse-Geisser	55.575	10.000	5.558		
	Huynh-Feldt	55.575	10.000	5.558		
	Lower-bound	55.575	10.000	5.558		

72-hour Energy Balance

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	6.286	1	6.286	.823	.386
	Greenhouse-Geisser	6.286	1.000	6.286	.823	.386
	Huynh-Feldt	6.286	1.000	6.286	.823	.386
	Lower-bound	6.286	1.000	6.286	.823	.386
Error(cond)	Sphericity Assumed	76.408	10	7.641		
	Greenhouse-Geisser	76.408	10.000	7.641		
	Huynh-Feldt	76.408	10.000	7.641		
	Lower-bound	76.408	10.000	7.641		

Subjective Appetite:

Days 1 and 2 (Maintenance days)

Hunger

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	127.841	1	127.841	.616	.451
	Greenhouse-Geisser	127.841	1.000	127.841	.616	.451
	Huynh-Feldt	127.841	1.000	127.841	.616	.451
	Lower-bound	127.841	1.000	127.841	.616	.451
Error(cond)	Sphericity Assumed	2073.909	10	207.391		
	Greenhouse-Geisser	2073.909	10.000	207.391		
	Huynh-Feldt	2073.909	10.000	207.391		
	Lower-bound	2073.909	10.000	207.391		
day	Sphericity Assumed	172.023	1	172.023	4.391	.063
	Greenhouse-Geisser	172.023	1.000	172.023	4.391	.063
	Huynh-Feldt	172.023	1.000	172.023	4.391	.063
	Lower-bound	172.023	1.000	172.023	4.391	.063
Error(day)	Sphericity Assumed	391.727	10	39.173		
	Greenhouse-Geisser	391.727	10.000	39.173		
	Huynh-Feldt	391.727	10.000	39.173		
	Lower-bound	391.727	10.000	39.173		
cond * day	Sphericity Assumed	14.205	1	14.205	.919	.360
	Greenhouse-Geisser	14.205	1.000	14.205	.919	.360
	Huynh-Feldt	14.205	1.000	14.205	.919	.360
	Lower-bound	14.205	1.000	14.205	.919	.360
Error(cond*day)	Sphericity Assumed	154.545	10	15.455		
	Greenhouse-Geisser	154.545	10.000	15.455		
	Huynh-Feldt	154.545	10.000	15.455		
	Lower-bound	154.545	10.000	15.455		

Fullness

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.091	1	.091	.000	.987
	Greenhouse-Geisser	.091	1.000	.091	.000	.987
	Huynh-Feldt	.091	1.000	.091	.000	.987
	Lower-bound	.091	1.000	.091	.000	.987
Error(cond)	Sphericity Assumed	3260.409	10	326.041		
	Greenhouse-Geisser	3260.409	10.000	326.041		
	Huynh-Feldt	3260.409	10.000	326.041		
	Lower-bound	3260.409	10.000	326.041		
day	Sphericity Assumed	255.364	1	255.364	2.340	.157
	Greenhouse-Geisser	255.364	1.000	255.364	2.340	.157
	Huynh-Feldt	255.364	1.000	255.364	2.340	.157
	Lower-bound	255.364	1.000	255.364	2.340	.157
Error(day)	Sphericity Assumed	1091.136	10	109.114		
	Greenhouse-Geisser	1091.136	10.000	109.114		
	Huynh-Feldt	1091.136	10.000	109.114		
	Lower-bound	1091.136	10.000	109.114		
cond * day	Sphericity Assumed	1.455	1	1.455	.030	.867
	Greenhouse-Geisser	1.455	1.000	1.455	.030	.867
	Huynh-Feldt	1.455	1.000	1.455	.030	.867
	Lower-bound	1.455	1.000	1.455	.030	.867
Error(cond*day)	Sphericity Assumed	493.045	10	49.305		
	Greenhouse-Geisser	493.045	10.000	49.305		
	Huynh-Feldt	493.045	10.000	49.305		
	Lower-bound	493.045	10.000	49.305		

Prospective Food Consumption

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	99.000	1	99.000	.337	.574
	Greenhouse-Geisser	99.000	1.000	99.000	.337	.574
	Huynh-Feldt	99.000	1.000	99.000	.337	.574
	Lower-bound	99.000	1.000	99.000	.337	.574
Error(cond)	Sphericity Assumed	2939.000	10	293.900		
	Greenhouse-Geisser	2939.000	10.000	293.900		
	Huynh-Feldt	2939.000	10.000	293.900		
	Lower-bound	2939.000	10.000	293.900		
day	Sphericity Assumed	192.364	1	192.364	3.745	.082
	Greenhouse-Geisser	192.364	1.000	192.364	3.745	.082
	Huynh-Feldt	192.364	1.000	192.364	3.745	.082
	Lower-bound	192.364	1.000	192.364	3.745	.082
Error(day)	Sphericity Assumed	513.636	10	51.364		
	Greenhouse-Geisser	513.636	10.000	51.364		
	Huynh-Feldt	513.636	10.000	51.364		
	Lower-bound	513.636	10.000	51.364		
cond * day	Sphericity Assumed	20.455	1	20.455	1.020	.336
	Greenhouse-Geisser	20.455	1.000	20.455	1.020	.336
	Huynh-Feldt	20.455	1.000	20.455	1.020	.336
	Lower-bound	20.455	1.000	20.455	1.020	.336
Error(cond*day)	Sphericity Assumed	200.545	10	20.055		
	Greenhouse-Geisser	200.545	10.000	20.055		
	Huynh-Feldt	200.545	10.000	20.055		
	Lower-bound	200.545	10.000	20.055		

Day 3 NSEP and SED Interventions

Hunger

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1222.545	1	1222.545	5.790	.037
	Greenhouse-Geisser	1222.545	1.000	1222.545	5.790	.037
	Huynh-Feldt	1222.545	1.000	1222.545	5.790	.037
	Lower-bound	1222.545	1.000	1222.545	5.790	.037
Error(cond)	Sphericity Assumed	2111.455	10	211.145		
	Greenhouse-Geisser	2111.455	10.000	211.145		
	Huynh-Feldt	2111.455	10.000	211.145		
	Lower-bound	2111.455	10.000	211.145		

Days 3, 4 and 5 (Intervention day and follow-up days)

Hunger

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	11.045	1	11.045	.729	.413
	Greenhouse-Geisser	11.045	1.000	11.045	.729	.413
	Huynh-Feldt	11.045	1.000	11.045	.729	.413
	Lower-bound	11.045	1.000	11.045	.729	.413
Error(cond)	Sphericity Assumed	151.455	10	15.145		
	Greenhouse-Geisser	151.455	10.000	15.145		
	Huynh-Feldt	151.455	10.000	15.145		
	Lower-bound	151.455	10.000	15.145		
days	Sphericity Assumed	3.909	2	1.955	.086	.918
	Greenhouse-Geisser	3.909	1.200	3.259	.086	.819
	Huynh-Feldt	3.909	1.272	3.073	.086	.832
	Lower-bound	3.909	1.000	3.909	.086	.776
Error(days)	Sphericity Assumed	456.091	20	22.805		
	Greenhouse-Geisser	456.091	11.996	38.019		
	Huynh-Feldt	456.091	12.722	35.850		
	Lower-bound	456.091	10.000	45.609		
cond * days	Sphericity Assumed	5.727	2	2.864	.152	.860
	Greenhouse-Geisser	5.727	1.756	3.261	.152	.834
	Huynh-Feldt	5.727	2.000	2.864	.152	.860
	Lower-bound	5.727	1.000	5.727	.152	.705
Error(cond*days)	Sphericity Assumed	376.273	20	18.814		
	Greenhouse-Geisser	376.273	17.565	21.422		
	Huynh-Feldt	376.273	20.000	18.814		
	Lower-bound	376.273	10.000	37.627		

Fullness

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	154.561	1	154.561	1.829	.206
	Greenhouse-Geisser	154.561	1.000	154.561	1.829	.206
	Huynh-Feldt	154.561	1.000	154.561	1.829	.206
	Lower-bound	154.561	1.000	154.561	1.829	.206
Error(cond)	Sphericity Assumed	844.939	10	84.494		
	Greenhouse-Geisser	844.939	10.000	84.494		
	Huynh-Feldt	844.939	10.000	84.494		
	Lower-bound	844.939	10.000	84.494		
days	Sphericity Assumed	31.030	2	15.515	.417	.665
	Greenhouse-Geisser	31.030	1.523	20.374	.417	.613
	Huynh-Feldt	31.030	1.740	17.829	.417	.638
	Lower-bound	31.030	1.000	31.030	.417	.533
Error(days)	Sphericity Assumed	744.970	20	37.248		
	Greenhouse-Geisser	744.970	15.231	48.912		
	Huynh-Feldt	744.970	17.405	42.803		
	Lower-bound	744.970	10.000	74.497		
cond * days	Sphericity Assumed	.121	2	.061	.002	.998
	Greenhouse-Geisser	.121	1.652	.073	.002	.995
	Huynh-Feldt	.121	1.938	.063	.002	.998
	Lower-bound	.121	1.000	.121	.002	.967
Error(cond*days)	Sphericity Assumed	689.879	20	34.494		
	Greenhouse-Geisser	689.879	16.525	41.748		
	Huynh-Feldt	689.879	19.380	35.597		
	Lower-bound	689.879	10.000	68.988		

Prospective Food Consumption

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	15.515	1	15.515	.790	.395
	Greenhouse-Geisser	15.515	1.000	15.515	.790	.395
	Huynh-Feldt	15.515	1.000	15.515	.790	.395
	Lower-bound	15.515	1.000	15.515	.790	.395
Error(cond)	Sphericity Assumed	196.485	10	19.648		
	Greenhouse-Geisser	196.485	10.000	19.648		
	Huynh-Feldt	196.485	10.000	19.648		
	Lower-bound	196.485	10.000	19.648		
days	Sphericity Assumed	12.758	2	6.379	.317	.732
	Greenhouse-Geisser	12.758	1.379	9.254	.317	.654
	Huynh-Feldt	12.758	1.527	8.355	.317	.675
	Lower-bound	12.758	1.000	12.758	.317	.586
Error(days)	Sphericity Assumed	402.576	20	20.129		
	Greenhouse-Geisser	402.576	13.786	29.201		
	Huynh-Feldt	402.576	15.270	26.364		
	Lower-bound	402.576	10.000	40.258		
cond * days	Sphericity Assumed	26.394	2	13.197	.768	.477
	Greenhouse-Geisser	26.394	1.664	15.865	.768	.457
	Huynh-Feldt	26.394	1.955	13.498	.768	.475
	Lower-bound	26.394	1.000	26.394	.768	.401
Error(cond*days)	Sphericity Assumed	343.606	20	17.180		
	Greenhouse-Geisser	343.606	16.637	20.653		
	Huynh-Feldt	343.606	19.553	17.573		
	Lower-bound	343.606	10.000	34.361		

Mood:

Days 1 and 2 (Maintenance days)

cond * day	Alert	Sphericity Assumed	188.205	1	188.205	10.008	.010
		Greenhouse-Geisser	188.205	1.000	188.205	10.008	.010
		Huynh-Feldt	188.205	1.000	188.205	10.008	.010
		Lower-bound	188.205	1.000	188.205	10.008	.010
	Active	Sphericity Assumed	300.568	1	300.568	17.012	.002
		Greenhouse-Geisser	300.568	1.000	300.568	17.012	.002
		Huynh-Feldt	300.568	1.000	300.568	17.012	.002
		Lower-bound	300.568	1.000	300.568	17.012	.002
Error(cond*day)	Alert	Sphericity Assumed	188.045	10	18.805		
		Greenhouse-Geisser	188.045	10.000	18.805		
		Huynh-Feldt	188.045	10.000	18.805		
		Lower-bound	188.045	10.000	18.805		
	Active	Sphericity Assumed	176.682	10	17.668		
		Greenhouse-Geisser	176.682	10.000	17.668		
		Huynh-Feldt	176.682	10.000	17.668		
		Lower-bound	176.682	10.000	17.668		

Day 3 NSEP and SED Interventions

Alertness

Univariate Tests

Source	Measure		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	alert	Sphericity Assumed	590.727	1	590.727	6.620	.028
		Greenhouse-Geisser	590.727	1.000	590.727	6.620	.028
		Huynh-Feldt	590.727	1.000	590.727	6.620	.028
		Lower-bound	590.727	1.000	590.727	6.620	.028
Error(cond)	alert	Sphericity Assumed	892.273	10	89.227		
		Greenhouse-Geisser	892.273	10.000	89.227		
		Huynh-Feldt	892.273	10.000	89.227		
		Lower-bound	892.273	10.000	89.227		

Days 3, 4 and 5 (Intervention day and follow-up days)

The ANOVA table was too large to include, however no significant findings were identified as discussed in the thesis.

CHAPTER 6

Between Participant Characteristics

ANOVA

age

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.500	1	2.500	2.480	.154
Within Groups	8.064	8	1.008		
Total	10.564	9			

ANOVA

height

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	1	.007	1.798	.217
Within Groups	.030	8	.004		
Total	.037	9			

ANOVA

weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.492	1	11.492	.230	.644
Within Groups	399.457	8	49.932		
Total	410.949	9			

ANOVA

BMI

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.604	1	9.604	3.878	.084
Within Groups	19.812	8	2.476		
Total	29.416	9			

ANOVA

percBF

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.676	1	.676	.066	.803
Within Groups	81.520	8	10.190		
Total	82.196	9			

ANOVA

maturityoffset

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.936	1	1.936	2.503	.152
Within Groups	6.188	8	.774		
Total	8.124	9			

ANOVA

dietaryrestraint

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.009	1	.009	.009	.925
Within Groups	7.712	8	.964		
Total	7.721	9			

ANOVA

vo2peak

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.625	1	.625	.049	.830
Within Groups	102.044	8	12.756		
Total	102.669	9			

ANOVA

activity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4718918	1	4718917.974	7.728	.024
Within Groups	4884864	8	610607.970		
Total	9603782	9			

Energy Expenditure:

Days 1 and 2 (Maintenance days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.016	1	.016	.311	.592
	Greenhouse-Geisser	.016	1.000	.016	.311	.592
	Huynh-Feldt	.016	1.000	.016	.311	.592
	Lower-bound	.016	1.000	.016	.311	.592
cond * group	Sphericity Assumed	.001	1	.001	.016	.903
	Greenhouse-Geisser	.001	1.000	.001	.016	.903
	Huynh-Feldt	.001	1.000	.001	.016	.903
	Lower-bound	.001	1.000	.001	.016	.903
Error(cond)	Sphericity Assumed	.412	8	.051		
	Greenhouse-Geisser	.412	8.000	.051		
	Huynh-Feldt	.412	8.000	.051		
	Lower-bound	.412	8.000	.051		
day	Sphericity Assumed	.773	1	.773	12.428	.008
	Greenhouse-Geisser	.773	1.000	.773	12.428	.008
	Huynh-Feldt	.773	1.000	.773	12.428	.008
	Lower-bound	.773	1.000	.773	12.428	.008
day * group	Sphericity Assumed	.005	1	.005	.085	.778
	Greenhouse-Geisser	.005	1.000	.005	.085	.778
	Huynh-Feldt	.005	1.000	.005	.085	.778
	Lower-bound	.005	1.000	.005	.085	.778
Error(day)	Sphericity Assumed	.497	8	.062		
	Greenhouse-Geisser	.497	8.000	.062		
	Huynh-Feldt	.497	8.000	.062		
	Lower-bound	.497	8.000	.062		
cond * day	Sphericity Assumed	.001	1	.001	.028	.871
	Greenhouse-Geisser	.001	1.000	.001	.028	.871
	Huynh-Feldt	.001	1.000	.001	.028	.871
	Lower-bound	.001	1.000	.001	.028	.871
cond * day * group	Sphericity Assumed	.000	1	.000	.004	.953
	Greenhouse-Geisser	.000	1.000	.000	.004	.953
	Huynh-Feldt	.000	1.000	.000	.004	.953
	Lower-bound	.000	1.000	.000	.004	.953
Error(cond*day)	Sphericity Assumed	.343	8	.043		
	Greenhouse-Geisser	.343	8.000	.043		
	Huynh-Feldt	.343	8.000	.043		
	Lower-bound	.343	8.000	.043		

Day 3 NSEP and SED Interventions

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	3.750	1	3.750	142.442	.000
	Greenhouse-Geisser	3.750	1.000	3.750	142.442	.000
	Huynh-Feldt	3.750	1.000	3.750	142.442	.000
	Lower-bound	3.750	1.000	3.750	142.442	.000
cond * group	Sphericity Assumed	.017	1	.017	.639	.447
	Greenhouse-Geisser	.017	1.000	.017	.639	.447
	Huynh-Feldt	.017	1.000	.017	.639	.447
	Lower-bound	.017	1.000	.017	.639	.447
Error(cond)	Sphericity Assumed	.211	8	.026		
	Greenhouse-Geisser	.211	8.000	.026		
	Huynh-Feldt	.211	8.000	.026		
	Lower-bound	.211	8.000	.026		

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.219	1	1.219	8.424	.020
	Greenhouse-Geisser	1.219	1.000	1.219	8.424	.020
	Huynh-Feldt	1.219	1.000	1.219	8.424	.020
	Lower-bound	1.219	1.000	1.219	8.424	.020
cond * group	Sphericity Assumed	.078	1	.078	.542	.483
	Greenhouse-Geisser	.078	1.000	.078	.542	.483
	Huynh-Feldt	.078	1.000	.078	.542	.483
	Lower-bound	.078	1.000	.078	.542	.483
Error(cond)	Sphericity Assumed	1.158	8	.145		
	Greenhouse-Geisser	1.158	8.000	.145		
	Huynh-Feldt	1.158	8.000	.145		
	Lower-bound	1.158	8.000	.145		
day	Sphericity Assumed	6.915	4	1.729	34.585	.000
	Greenhouse-Geisser	6.915	2.658	2.601	34.585	.000
	Huynh-Feldt	6.915	4.000	1.729	34.585	.000
	Lower-bound	6.915	1.000	6.915	34.585	.000
day * group	Sphericity Assumed	.055	4	.014	.277	.891
	Greenhouse-Geisser	.055	2.658	.021	.277	.819
	Huynh-Feldt	.055	4.000	.014	.277	.891
	Lower-bound	.055	1.000	.055	.277	.613
Error(day)	Sphericity Assumed	1.600	32	.050		
	Greenhouse-Geisser	1.600	21.267	.075		
	Huynh-Feldt	1.600	32.000	.050		
	Lower-bound	1.600	8.000	.200		
cond * day	Sphericity Assumed	4.319	4	1.080	26.293	.000
	Greenhouse-Geisser	4.319	2.432	1.776	26.293	.000
	Huynh-Feldt	4.319	4.000	1.080	26.293	.000
	Lower-bound	4.319	1.000	4.319	26.293	.001
cond * day * group	Sphericity Assumed	.092	4	.023	.558	.695
	Greenhouse-Geisser	.092	2.432	.038	.558	.614
	Huynh-Feldt	.092	4.000	.023	.558	.695
	Lower-bound	.092	1.000	.092	.558	.476
Error(cond*day)	Sphericity Assumed	1.314	32	.041		
	Greenhouse-Geisser	1.314	19.459	.068		
	Huynh-Feldt	1.314	32.000	.041		
	Lower-bound	1.314	8.000	.164		

Day 3 Energy Expenditure NSEP versus SED

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	5.512	1	5.512	50.829	.000
	Greenhouse-Geisser	5.512	1.000	5.512	50.829	.000
	Huynh-Feldt	5.512	1.000	5.512	50.829	.000
	Lower-bound	5.512	1.000	5.512	50.829	.000
cond * group	Sphericity Assumed	.006	1	.006	.060	.813
	Greenhouse-Geisser	.006	1.000	.006	.060	.813
	Huynh-Feldt	.006	1.000	.006	.060	.813
	Lower-bound	.006	1.000	.006	.060	.813
Error(cond)	Sphericity Assumed	.868	8	.108		
	Greenhouse-Geisser	.868	8.000	.108		
	Huynh-Feldt	.868	8.000	.108		
	Lower-bound	.868	8.000	.108		

5-day Energy Expenditure

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	6.094	1	6.094	8.424	.020
	Greenhouse-Geisser	6.094	1.000	6.094	8.424	.020
	Huynh-Feldt	6.094	1.000	6.094	8.424	.020
	Lower-bound	6.094	1.000	6.094	8.424	.020
cond * group	Sphericity Assumed	.392	1	.392	.542	.483
	Greenhouse-Geisser	.392	1.000	.392	.542	.483
	Huynh-Feldt	.392	1.000	.392	.542	.483
	Lower-bound	.392	1.000	.392	.542	.483
Error(cond)	Sphericity Assumed	5.788	8	.723		
	Greenhouse-Geisser	5.788	8.000	.723		
	Huynh-Feldt	5.788	8.000	.723		
	Lower-bound	5.788	8.000	.723		

Energy Intake:

Days 1 and 2 (Maintenance days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	4.090	1	4.090	1.537	.250
	Greenhouse-Geisser	4.090	1.000	4.090	1.537	.250
	Huynh-Feldt	4.090	1.000	4.090	1.537	.250
	Lower-bound	4.090	1.000	4.090	1.537	.250
cond * participant	Sphericity Assumed	.195	1	.195	.073	.794
	Greenhouse-Geisser	.195	1.000	.195	.073	.794
	Huynh-Feldt	.195	1.000	.195	.073	.794
	Lower-bound	.195	1.000	.195	.073	.794
Error(cond)	Sphericity Assumed	21.280	8	2.660		
	Greenhouse-Geisser	21.280	8.000	2.660		
	Huynh-Feldt	21.280	8.000	2.660		
	Lower-bound	21.280	8.000	2.660		
day	Sphericity Assumed	28.375	1	28.375	2.635	.143
	Greenhouse-Geisser	28.375	1.000	28.375	2.635	.143
	Huynh-Feldt	28.375	1.000	28.375	2.635	.143
	Lower-bound	28.375	1.000	28.375	2.635	.143
day * participant	Sphericity Assumed	1.923	1	1.923	.179	.684
	Greenhouse-Geisser	1.923	1.000	1.923	.179	.684
	Huynh-Feldt	1.923	1.000	1.923	.179	.684
	Lower-bound	1.923	1.000	1.923	.179	.684
Error(day)	Sphericity Assumed	86.157	8	10.770		
	Greenhouse-Geisser	86.157	8.000	10.770		
	Huynh-Feldt	86.157	8.000	10.770		
	Lower-bound	86.157	8.000	10.770		
cond * day	Sphericity Assumed	.491	1	.491	.672	.436
	Greenhouse-Geisser	.491	1.000	.491	.672	.436
	Huynh-Feldt	.491	1.000	.491	.672	.436
	Lower-bound	.491	1.000	.491	.672	.436
cond * day * participant	Sphericity Assumed	.915	1	.915	1.254	.295
	Greenhouse-Geisser	.915	1.000	.915	1.254	.295
	Huynh-Feldt	.915	1.000	.915	1.254	.295
	Lower-bound	.915	1.000	.915	1.254	.295
Error(cond*day)	Sphericity Assumed	5.838	8	.730		
	Greenhouse-Geisser	5.838	8.000	.730		
	Huynh-Feldt	5.838	8.000	.730		
	Lower-bound	5.838	8.000	.730		

Day 3 Test Meal (Intervention day)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.038	1	.038	.219	.652
	Greenhouse-Geisser	.038	1.000	.038	.219	.652
	Huynh-Feldt	.038	1.000	.038	.219	.652
	Lower-bound	.038	1.000	.038	.219	.652
cond * participant	Sphericity Assumed	.133	1	.133	.769	.406
	Greenhouse-Geisser	.133	1.000	.133	.769	.406
	Huynh-Feldt	.133	1.000	.133	.769	.406
	Lower-bound	.133	1.000	.133	.769	.406
Error(cond)	Sphericity Assumed	1.383	8	.173		
	Greenhouse-Geisser	1.383	8.000	.173		
	Huynh-Feldt	1.383	8.000	.173		
	Lower-bound	1.383	8.000	.173		

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.388	1	.388	.115	.743
	Greenhouse-Geisser	.388	1.000	.388	.115	.743
	Huynh-Feldt	.388	1.000	.388	.115	.743
	Lower-bound	.388	1.000	.388	.115	.743
cond * participant	Sphericity Assumed	11.069	1	11.069	3.281	.108
	Greenhouse-Geisser	11.069	1.000	11.069	3.281	.108
	Huynh-Feldt	11.069	1.000	11.069	3.281	.108
	Lower-bound	11.069	1.000	11.069	3.281	.108
Error(cond)	Sphericity Assumed	26.986	8	3.373		
	Greenhouse-Geisser	26.986	8.000	3.373		
	Huynh-Feldt	26.986	8.000	3.373		
	Lower-bound	26.986	8.000	3.373		
day	Sphericity Assumed	42.964	4	10.741	4.280	.007
	Greenhouse-Geisser	42.964	2.881	14.914	4.280	.016
	Huynh-Feldt	42.964	4.000	10.741	4.280	.007
	Lower-bound	42.964	1.000	42.964	4.280	.072
day * participant	Sphericity Assumed	9.206	4	2.302	.917	.466
	Greenhouse-Geisser	9.206	2.881	3.196	.917	.445
	Huynh-Feldt	9.206	4.000	2.302	.917	.466
	Lower-bound	9.206	1.000	9.206	.917	.366
Error(day)	Sphericity Assumed	80.303	32	2.509		
	Greenhouse-Geisser	80.303	23.046	3.484		
	Huynh-Feldt	80.303	32.000	2.509		
	Lower-bound	80.303	8.000	10.038		
cond * day	Sphericity Assumed	16.828	4	4.207	1.656	.185
	Greenhouse-Geisser	16.828	2.762	6.091	1.656	.208
	Huynh-Feldt	16.828	4.000	4.207	1.656	.185
	Lower-bound	16.828	1.000	16.828	1.656	.234
cond * day * participant	Sphericity Assumed	38.550	4	9.637	3.793	.012
	Greenhouse-Geisser	38.550	2.762	13.955	3.793	.027
	Huynh-Feldt	38.550	4.000	9.637	3.793	.012
	Lower-bound	38.550	1.000	38.550	3.793	.087
Error(cond*day)	Sphericity Assumed	81.313	32	2.541		
	Greenhouse-Geisser	81.313	22.100	3.679		
	Huynh-Feldt	81.313	32.000	2.541		
	Lower-bound	81.313	8.000	10.164		

Macronutrient Intake:

Days 1 and 2 (Maintenance days)

Fat

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	2.357	1	2.357	.043	.841
	Greenhouse-Geisser	2.357	1.000	2.357	.043	.841
	Huynh-Feldt	2.357	1.000	2.357	.043	.841
	Lower-bound	2.357	1.000	2.357	.043	.841
cond * participant	Sphericity Assumed	.057	1	.057	.001	.975
	Greenhouse-Geisser	.057	1.000	.057	.001	.975
	Huynh-Feldt	.057	1.000	.057	.001	.975
	Lower-bound	.057	1.000	.057	.001	.975
Error(cond)	Sphericity Assumed	441.206	8	55.151		
	Greenhouse-Geisser	441.206	8.000	55.151		
	Huynh-Feldt	441.206	8.000	55.151		
	Lower-bound	441.206	8.000	55.151		
day	Sphericity Assumed	3.142	1	3.142	.114	.744
	Greenhouse-Geisser	3.142	1.000	3.142	.114	.744
	Huynh-Feldt	3.142	1.000	3.142	.114	.744
	Lower-bound	3.142	1.000	3.142	.114	.744
day * participant	Sphericity Assumed	.897	1	.897	.033	.861
	Greenhouse-Geisser	.897	1.000	.897	.033	.861
	Huynh-Feldt	.897	1.000	.897	.033	.861
	Lower-bound	.897	1.000	.897	.033	.861
Error(day)	Sphericity Assumed	219.511	8	27.439		
	Greenhouse-Geisser	219.511	8.000	27.439		
	Huynh-Feldt	219.511	8.000	27.439		
	Lower-bound	219.511	8.000	27.439		
cond * day	Sphericity Assumed	29.705	1	29.705	1.785	.218
	Greenhouse-Geisser	29.705	1.000	29.705	1.785	.218
	Huynh-Feldt	29.705	1.000	29.705	1.785	.218
	Lower-bound	29.705	1.000	29.705	1.785	.218
cond * day * participant	Sphericity Assumed	15.838	1	15.838	.952	.358
	Greenhouse-Geisser	15.838	1.000	15.838	.952	.358
	Huynh-Feldt	15.838	1.000	15.838	.952	.358
	Lower-bound	15.838	1.000	15.838	.952	.358
Error(cond*day)	Sphericity Assumed	133.136	8	16.642		
	Greenhouse-Geisser	133.136	8.000	16.642		
	Huynh-Feldt	133.136	8.000	16.642		
	Lower-bound	133.136	8.000	16.642		

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	7.735	1	7.735	.061	.810
	Greenhouse-Geisser	7.735	1.000	7.735	.061	.810
	Huynh-Feldt	7.735	1.000	7.735	.061	.810
	Lower-bound	7.735	1.000	7.735	.061	.810
cond * participant	Sphericity Assumed	1.509	1	1.509	.012	.916
	Greenhouse-Geisser	1.509	1.000	1.509	.012	.916
	Huynh-Feldt	1.509	1.000	1.509	.012	.916
	Lower-bound	1.509	1.000	1.509	.012	.916
Error(cond)	Sphericity Assumed	1007.389	8	125.924		
	Greenhouse-Geisser	1007.389	8.000	125.924		
	Huynh-Feldt	1007.389	8.000	125.924		
	Lower-bound	1007.389	8.000	125.924		
day	Sphericity Assumed	227.386	1	227.386	1.640	.236
	Greenhouse-Geisser	227.386	1.000	227.386	1.640	.236
	Huynh-Feldt	227.386	1.000	227.386	1.640	.236
	Lower-bound	227.386	1.000	227.386	1.640	.236
day * participant	Sphericity Assumed	48.071	1	48.071	.347	.572
	Greenhouse-Geisser	48.071	1.000	48.071	.347	.572
	Huynh-Feldt	48.071	1.000	48.071	.347	.572
	Lower-bound	48.071	1.000	48.071	.347	.572
Error(day)	Sphericity Assumed	1109.429	8	138.679		
	Greenhouse-Geisser	1109.429	8.000	138.679		
	Huynh-Feldt	1109.429	8.000	138.679		
	Lower-bound	1109.429	8.000	138.679		
cond * day	Sphericity Assumed	5.891	1	5.891	.589	.465
	Greenhouse-Geisser	5.891	1.000	5.891	.589	.465
	Huynh-Feldt	5.891	1.000	5.891	.589	.465
	Lower-bound	5.891	1.000	5.891	.589	.465
cond * day * participant	Sphericity Assumed	17.174	1	17.174	1.717	.226
	Greenhouse-Geisser	17.174	1.000	17.174	1.717	.226
	Huynh-Feldt	17.174	1.000	17.174	1.717	.226
	Lower-bound	17.174	1.000	17.174	1.717	.226
Error(cond*day)	Sphericity Assumed	80.032	8	10.004		
	Greenhouse-Geisser	80.032	8.000	10.004		
	Huynh-Feldt	80.032	8.000	10.004		
	Lower-bound	80.032	8.000	10.004		

Protein

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	18.632	1	18.632	.838	.387
	Greenhouse-Geisser	18.632	1.000	18.632	.838	.387
	Huynh-Feldt	18.632	1.000	18.632	.838	.387
	Lower-bound	18.632	1.000	18.632	.838	.387
cond * participant	Sphericity Assumed	.992	1	.992	.045	.838
	Greenhouse-Geisser	.992	1.000	.992	.045	.838
	Huynh-Feldt	.992	1.000	.992	.045	.838
	Lower-bound	.992	1.000	.992	.045	.838
Error(cond)	Sphericity Assumed	177.807	8	22.226		
	Greenhouse-Geisser	177.807	8.000	22.226		
	Huynh-Feldt	177.807	8.000	22.226		
	Lower-bound	177.807	8.000	22.226		
day	Sphericity Assumed	176.904	1	176.904	2.000	.195
	Greenhouse-Geisser	176.904	1.000	176.904	2.000	.195
	Huynh-Feldt	176.904	1.000	176.904	2.000	.195
	Lower-bound	176.904	1.000	176.904	2.000	.195
day * participant	Sphericity Assumed	62.101	1	62.101	.702	.426
	Greenhouse-Geisser	62.101	1.000	62.101	.702	.426
	Huynh-Feldt	62.101	1.000	62.101	.702	.426
	Lower-bound	62.101	1.000	62.101	.702	.426
Error(day)	Sphericity Assumed	707.562	8	88.445		
	Greenhouse-Geisser	707.562	8.000	88.445		
	Huynh-Feldt	707.562	8.000	88.445		
	Lower-bound	707.562	8.000	88.445		
cond * day	Sphericity Assumed	9.063	1	9.063	1.259	.294
	Greenhouse-Geisser	9.063	1.000	9.063	1.259	.294
	Huynh-Feldt	9.063	1.000	9.063	1.259	.294
	Lower-bound	9.063	1.000	9.063	1.259	.294
cond * day * participant	Sphericity Assumed	.025	1	.025	.003	.954
	Greenhouse-Geisser	.025	1.000	.025	.003	.954
	Huynh-Feldt	.025	1.000	.025	.003	.954
	Lower-bound	.025	1.000	.025	.003	.954
Error(cond*day)	Sphericity Assumed	57.591	8	7.199		
	Greenhouse-Geisser	57.591	8.000	7.199		
	Huynh-Feldt	57.591	8.000	7.199		
	Lower-bound	57.591	8.000	7.199		

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Fat

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	81.776	1	81.776	14.412	.005
	Greenhouse-Geisser	81.776	1.000	81.776	14.412	.005
	Huynh-Feldt	81.776	1.000	81.776	14.412	.005
	Lower-bound	81.776	1.000	81.776	14.412	.005
cond * participant	Sphericity Assumed	14.846	1	14.846	2.616	.144
	Greenhouse-Geisser	14.846	1.000	14.846	2.616	.144
	Huynh-Feldt	14.846	1.000	14.846	2.616	.144
	Lower-bound	14.846	1.000	14.846	2.616	.144
Error(cond)	Sphericity Assumed	45.394	8	5.674		
	Greenhouse-Geisser	45.394	8.000	5.674		
	Huynh-Feldt	45.394	8.000	5.674		
	Lower-bound	45.394	8.000	5.674		
day	Sphericity Assumed	477.295	4	119.324	6.746	.000
	Greenhouse-Geisser	477.295	2.671	178.711	6.746	.003
	Huynh-Feldt	477.295	4.000	119.324	6.746	.000
	Lower-bound	477.295	1.000	477.295	6.746	.032
day * participant	Sphericity Assumed	35.014	4	8.753	.495	.740
	Greenhouse-Geisser	35.014	2.671	13.110	.495	.669
	Huynh-Feldt	35.014	4.000	8.753	.495	.740
	Lower-bound	35.014	1.000	35.014	.495	.502
Error(day)	Sphericity Assumed	565.994	32	17.687		
	Greenhouse-Geisser	565.994	21.366	26.490		
	Huynh-Feldt	565.994	32.000	17.687		
	Lower-bound	565.994	8.000	70.749		
cond * day	Sphericity Assumed	139.178	4	34.795	1.363	.268
	Greenhouse-Geisser	139.178	2.145	64.898	1.363	.283
	Huynh-Feldt	139.178	3.321	41.909	1.363	.275
	Lower-bound	139.178	1.000	139.178	1.363	.277
cond * day * participant	Sphericity Assumed	88.541	4	22.135	.867	.494
	Greenhouse-Geisser	88.541	2.145	41.286	.867	.445
	Huynh-Feldt	88.541	3.321	26.661	.867	.480
	Lower-bound	88.541	1.000	88.541	.867	.379
Error(cond*day)	Sphericity Assumed	816.669	32	25.521		
	Greenhouse-Geisser	816.669	17.157	47.601		
	Huynh-Feldt	816.669	26.568	30.739		
	Lower-bound	816.669	8.000	102.084		

Energy Balance:

Days 1 and 2 (Maintenance days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	3.329	1	3.329	1.373	.275
	Greenhouse-Geisser	3.329	1.000	3.329	1.373	.275
	Huynh-Feldt	3.329	1.000	3.329	1.373	.275
	Lower-bound	3.329	1.000	3.329	1.373	.275
cond * participant	Sphericity Assumed	.234	1	.234	.097	.764
	Greenhouse-Geisser	.234	1.000	.234	.097	.764
	Huynh-Feldt	.234	1.000	.234	.097	.764
	Lower-bound	.234	1.000	.234	.097	.764
Error(cond)	Sphericity Assumed	19.393	8	2.424		
	Greenhouse-Geisser	19.393	8.000	2.424		
	Huynh-Feldt	19.393	8.000	2.424		
	Lower-bound	19.393	8.000	2.424		
day	Sphericity Assumed	19.155	1	19.155	1.786	.218
	Greenhouse-Geisser	19.155	1.000	19.155	1.786	.218
	Huynh-Feldt	19.155	1.000	19.155	1.786	.218
	Lower-bound	19.155	1.000	19.155	1.786	.218
day * participant	Sphericity Assumed	2.343	1	2.343	.218	.653
	Greenhouse-Geisser	2.343	1.000	2.343	.218	.653
	Huynh-Feldt	2.343	1.000	2.343	.218	.653
	Lower-bound	2.343	1.000	2.343	.218	.653
Error(day)	Sphericity Assumed	85.786	8	10.723		
	Greenhouse-Geisser	85.786	8.000	10.723		
	Huynh-Feldt	85.786	8.000	10.723		
	Lower-bound	85.786	8.000	10.723		
cond * day	Sphericity Assumed	.650	1	.650	1.043	.337
	Greenhouse-Geisser	.650	1.000	.650	1.043	.337
	Huynh-Feldt	.650	1.000	.650	1.043	.337
	Lower-bound	.650	1.000	.650	1.043	.337
cond * day * participant	Sphericity Assumed	1.030	1	1.030	1.652	.235
	Greenhouse-Geisser	1.030	1.000	1.030	1.652	.235
	Huynh-Feldt	1.030	1.000	1.030	1.652	.235
	Lower-bound	1.030	1.000	1.030	1.652	.235
Error(cond*day)	Sphericity Assumed	4.989	8	.624		
	Greenhouse-Geisser	4.989	8.000	.624		
	Huynh-Feldt	4.989	8.000	.624		
	Lower-bound	4.989	8.000	.624		

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.419	1	.419	.149	.709
	Greenhouse-Geisser	.419	1.000	.419	.149	.709
	Huynh-Feldt	.419	1.000	.419	.149	.709
	Lower-bound	.419	1.000	.419	.149	.709
cond * participant	Sphericity Assumed	10.323	1	10.323	3.680	.091
	Greenhouse-Geisser	10.323	1.000	10.323	3.680	.091
	Huynh-Feldt	10.323	1.000	10.323	3.680	.091
	Lower-bound	10.323	1.000	10.323	3.680	.091
Error(cond)	Sphericity Assumed	22.441	8	2.805		
	Greenhouse-Geisser	22.441	8.000	2.805		
	Huynh-Feldt	22.441	8.000	2.805		
	Lower-bound	22.441	8.000	2.805		
day	Sphericity Assumed	25.089	4	6.272	2.591	.055
	Greenhouse-Geisser	25.089	2.807	8.938	2.591	.081
	Huynh-Feldt	25.089	4.000	6.272	2.591	.055
	Lower-bound	25.089	1.000	25.089	2.591	.146
day * participant	Sphericity Assumed	8.109	4	2.027	.838	.512
	Greenhouse-Geisser	8.109	2.807	2.889	.838	.481
	Huynh-Feldt	8.109	4.000	2.027	.838	.512
	Lower-bound	8.109	1.000	8.109	.838	.387
Error(day)	Sphericity Assumed	77.454	32	2.420		
	Greenhouse-Geisser	77.454	22.455	3.449		
	Huynh-Feldt	77.454	32.000	2.420		
	Lower-bound	77.454	8.000	9.682		
cond * day	Sphericity Assumed	20.875	4	5.219	1.958	.125
	Greenhouse-Geisser	20.875	2.728	7.651	1.958	.154
	Huynh-Feldt	20.875	4.000	5.219	1.958	.125
	Lower-bound	20.875	1.000	20.875	1.958	.199
cond * day * participant	Sphericity Assumed	33.255	4	8.314	3.119	.028
	Greenhouse-Geisser	33.255	2.728	12.188	3.119	.051
	Huynh-Feldt	33.255	4.000	8.314	3.119	.028
	Lower-bound	33.255	1.000	33.255	3.119	.115
Error(cond*day)	Sphericity Assumed	85.308	32	2.666		
	Greenhouse-Geisser	85.308	21.827	3.908		
	Huynh-Feldt	85.308	32.000	2.666		
	Lower-bound	85.308	8.000	10.664		

2-day and 4-day Energy Balance

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	3.707	1	3.707	.658	.441
	Greenhouse-Geisser	3.707	1.000	3.707	.658	.441
	Huynh-Feldt	3.707	1.000	3.707	.658	.441
	Lower-bound	3.707	1.000	3.707	.658	.441
cond * participant	Sphericity Assumed	26.243	1	26.243	4.658	.063
	Greenhouse-Geisser	26.243	1.000	26.243	4.658	.063
	Huynh-Feldt	26.243	1.000	26.243	4.658	.063
	Lower-bound	26.243	1.000	26.243	4.658	.063
Error(cond)	Sphericity Assumed	45.073	8	5.634		
	Greenhouse-Geisser	45.073	8.000	5.634		
	Huynh-Feldt	45.073	8.000	5.634		
	Lower-bound	45.073	8.000	5.634		

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.496	1	1.496	.124	.734
	Greenhouse-Geisser	1.496	1.000	1.496	.124	.734
	Huynh-Feldt	1.496	1.000	1.496	.124	.734
	Lower-bound	1.496	1.000	1.496	.124	.734
cond * participant	Sphericity Assumed	55.411	1	55.411	4.576	.065
	Greenhouse-Geisser	55.411	1.000	55.411	4.576	.065
	Huynh-Feldt	55.411	1.000	55.411	4.576	.065
	Lower-bound	55.411	1.000	55.411	4.576	.065
Error(cond)	Sphericity Assumed	96.883	8	12.110		
	Greenhouse-Geisser	96.883	8.000	12.110		
	Huynh-Feldt	96.883	8.000	12.110		
	Lower-bound	96.883	8.000	12.110		

Subjective Appetite:

Days 1 and 2 (Maintenance days)

Hunger

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	57.600	1	57.600	.462	.516
	Greenhouse-Geisser	57.600	1.000	57.600	.462	.516
	Huynh-Feldt	57.600	1.000	57.600	.462	.516
	Lower-bound	57.600	1.000	57.600	.462	.516
cond * group	Sphericity Assumed	270.400	1	270.400	2.171	.179
	Greenhouse-Geisser	270.400	1.000	270.400	2.171	.179
	Huynh-Feldt	270.400	1.000	270.400	2.171	.179
	Lower-bound	270.400	1.000	270.400	2.171	.179
Error(cond)	Sphericity Assumed	996.500	8	124.563		
	Greenhouse-Geisser	996.500	8.000	124.563		
	Huynh-Feldt	996.500	8.000	124.563		
	Lower-bound	996.500	8.000	124.563		
day	Sphericity Assumed	.400	1	.400	.010	.923
	Greenhouse-Geisser	.400	1.000	.400	.010	.923
	Huynh-Feldt	.400	1.000	.400	.010	.923
	Lower-bound	.400	1.000	.400	.010	.923
day * group	Sphericity Assumed	102.400	1	102.400	2.531	.150
	Greenhouse-Geisser	102.400	1.000	102.400	2.531	.150
	Huynh-Feldt	102.400	1.000	102.400	2.531	.150
	Lower-bound	102.400	1.000	102.400	2.531	.150
Error(day)	Sphericity Assumed	323.700	8	40.463		
	Greenhouse-Geisser	323.700	8.000	40.463		
	Huynh-Feldt	323.700	8.000	40.463		
	Lower-bound	323.700	8.000	40.463		
cond * day	Sphericity Assumed	8.100	1	8.100	.111	.748
	Greenhouse-Geisser	8.100	1.000	8.100	.111	.748
	Huynh-Feldt	8.100	1.000	8.100	.111	.748
	Lower-bound	8.100	1.000	8.100	.111	.748
cond * day * group	Sphericity Assumed	84.100	1	84.100	1.149	.315
	Greenhouse-Geisser	84.100	1.000	84.100	1.149	.315
	Huynh-Feldt	84.100	1.000	84.100	1.149	.315
	Lower-bound	84.100	1.000	84.100	1.149	.315
Error(cond*day)	Sphericity Assumed	585.300	8	73.163		
	Greenhouse-Geisser	585.300	8.000	73.163		
	Huynh-Feldt	585.300	8.000	73.163		
	Lower-bound	585.300	8.000	73.163		

Fullness

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	108.900	1	108.900	1.137	.317
	Greenhouse-Geisser	108.900	1.000	108.900	1.137	.317
	Huynh-Feldt	108.900	1.000	108.900	1.137	.317
	Lower-bound	108.900	1.000	108.900	1.137	.317
cond * group	Sphericity Assumed	3.600	1	3.600	.038	.851
	Greenhouse-Geisser	3.600	1.000	3.600	.038	.851
	Huynh-Feldt	3.600	1.000	3.600	.038	.851
	Lower-bound	3.600	1.000	3.600	.038	.851
Error(cond)	Sphericity Assumed	766.500	8	95.813		
	Greenhouse-Geisser	766.500	8.000	95.813		
	Huynh-Feldt	766.500	8.000	95.813		
	Lower-bound	766.500	8.000	95.813		
day	Sphericity Assumed	22.500	1	22.500	.132	.726
	Greenhouse-Geisser	22.500	1.000	22.500	.132	.726
	Huynh-Feldt	22.500	1.000	22.500	.132	.726
	Lower-bound	22.500	1.000	22.500	.132	.726
day * group	Sphericity Assumed	14.400	1	14.400	.084	.779
	Greenhouse-Geisser	14.400	1.000	14.400	.084	.779
	Huynh-Feldt	14.400	1.000	14.400	.084	.779
	Lower-bound	14.400	1.000	14.400	.084	.779
Error(day)	Sphericity Assumed	1365.100	8	170.638		
	Greenhouse-Geisser	1365.100	8.000	170.638		
	Huynh-Feldt	1365.100	8.000	170.638		
	Lower-bound	1365.100	8.000	170.638		
cond * day	Sphericity Assumed	14.400	1	14.400	.126	.731
	Greenhouse-Geisser	14.400	1.000	14.400	.126	.731
	Huynh-Feldt	14.400	1.000	14.400	.126	.731
	Lower-bound	14.400	1.000	14.400	.126	.731
cond * day * group	Sphericity Assumed	122.500	1	122.500	1.074	.330
	Greenhouse-Geisser	122.500	1.000	122.500	1.074	.330
	Huynh-Feldt	122.500	1.000	122.500	1.074	.330
	Lower-bound	122.500	1.000	122.500	1.074	.330
Error(cond*day)	Sphericity Assumed	912.100	8	114.013		
	Greenhouse-Geisser	912.100	8.000	114.013		
	Huynh-Feldt	912.100	8.000	114.013		
	Lower-bound	912.100	8.000	114.013		

Prospective Food Consumption

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	38.025	1	38.025	.283	.609
	Greenhouse-Geisser	38.025	1.000	38.025	.283	.609
	Huynh-Feldt	38.025	1.000	38.025	.283	.609
	Lower-bound	38.025	1.000	38.025	.283	.609
cond * group	Sphericity Assumed	112.225	1	112.225	.836	.387
	Greenhouse-Geisser	112.225	1.000	112.225	.836	.387
	Huynh-Feldt	112.225	1.000	112.225	.836	.387
	Lower-bound	112.225	1.000	112.225	.836	.387
Error(cond)	Sphericity Assumed	1074.500	8	134.313		
	Greenhouse-Geisser	1074.500	8.000	134.313		
	Huynh-Feldt	1074.500	8.000	134.313		
	Lower-bound	1074.500	8.000	134.313		
day	Sphericity Assumed	13.225	1	13.225	.165	.695
	Greenhouse-Geisser	13.225	1.000	13.225	.165	.695
	Huynh-Feldt	13.225	1.000	13.225	.165	.695
	Lower-bound	13.225	1.000	13.225	.165	.695
day * group	Sphericity Assumed	81.225	1	81.225	1.012	.344
	Greenhouse-Geisser	81.225	1.000	81.225	1.012	.344
	Huynh-Feldt	81.225	1.000	81.225	1.012	.344
	Lower-bound	81.225	1.000	81.225	1.012	.344
Error(day)	Sphericity Assumed	642.300	8	80.288		
	Greenhouse-Geisser	642.300	8.000	80.288		
	Huynh-Feldt	642.300	8.000	80.288		
	Lower-bound	642.300	8.000	80.288		
cond * day	Sphericity Assumed	2.025	1	2.025	.038	.851
	Greenhouse-Geisser	2.025	1.000	2.025	.038	.851
	Huynh-Feldt	2.025	1.000	2.025	.038	.851
	Lower-bound	2.025	1.000	2.025	.038	.851
cond * day * group	Sphericity Assumed	.025	1	.025	.000	.983
	Greenhouse-Geisser	.025	1.000	.025	.000	.983
	Huynh-Feldt	.025	1.000	.025	.000	.983
	Lower-bound	.025	1.000	.025	.000	.983
Error(cond*day)	Sphericity Assumed	430.700	8	53.838		
	Greenhouse-Geisser	430.700	8.000	53.838		
	Huynh-Feldt	430.700	8.000	53.838		
	Lower-bound	430.700	8.000	53.838		

Day 3 NSEP and SED Interventions

Hunger

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	233.472	1	233.472	.203	.664
	Greenhouse-Geisser	233.472	1.000	233.472	.203	.664
	Huynh-Feldt	233.472	1.000	233.472	.203	.664
	Lower-bound	233.472	1.000	233.472	.203	.664
cond * group	Sphericity Assumed	2.006	1	2.006	.002	.968
	Greenhouse-Geisser	2.006	1.000	2.006	.002	.968
	Huynh-Feldt	2.006	1.000	2.006	.002	.968
	Lower-bound	2.006	1.000	2.006	.002	.968
Error(cond)	Sphericity Assumed	9192.022	8	1149.003		
	Greenhouse-Geisser	9192.022	8.000	1149.003		
	Huynh-Feldt	9192.022	8.000	1149.003		
	Lower-bound	9192.022	8.000	1149.003		
time	Sphericity Assumed	7752.778	8	969.097	6.945	.000
	Greenhouse-Geisser	7752.778	2.493	3109.361	6.945	.003
	Huynh-Feldt	7752.778	4.165	1861.530	6.945	.000
	Lower-bound	7752.778	1.000	7752.778	6.945	.030
time * group	Sphericity Assumed	1893.111	8	236.639	1.696	.117
	Greenhouse-Geisser	1893.111	2.493	759.259	1.696	.205
	Huynh-Feldt	1893.111	4.165	454.557	1.696	.172
	Lower-bound	1893.111	1.000	1893.111	1.696	.229
Error(time)	Sphericity Assumed	8931.111	64	139.549		
	Greenhouse-Geisser	8931.111	19.947	447.744		
	Huynh-Feldt	8931.111	33.318	268.058		
	Lower-bound	8931.111	8.000	1116.389		
cond * time	Sphericity Assumed	1799.578	8	224.947	1.417	.207
	Greenhouse-Geisser	1799.578	2.659	676.815	1.417	.266
	Huynh-Feldt	1799.578	4.604	390.897	1.417	.244
	Lower-bound	1799.578	1.000	1799.578	1.417	.268
cond * time * group	Sphericity Assumed	937.644	8	117.206	.738	.658
	Greenhouse-Geisser	937.644	2.659	352.645	.738	.526
	Huynh-Feldt	937.644	4.604	203.672	.738	.589
	Lower-bound	937.644	1.000	937.644	.738	.415
Error(cond*time)	Sphericity Assumed	10161.778	64	158.778		
	Greenhouse-Geisser	10161.778	21.271	477.726		
	Huynh-Feldt	10161.778	36.830	275.913		
	Lower-bound	10161.778	8.000	1270.222		

Prospective Food Consumption

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	500.000	1	500.000	.901	.370
	Greenhouse-Geisser	500.000	1.000	500.000	.901	.370
	Huynh-Feldt	500.000	1.000	500.000	.901	.370
	Lower-bound	500.000	1.000	500.000	.901	.370
cond * group	Sphericity Assumed	128.356	1	128.356	.231	.643
	Greenhouse-Geisser	128.356	1.000	128.356	.231	.643
	Huynh-Feldt	128.356	1.000	128.356	.231	.643
	Lower-bound	128.356	1.000	128.356	.231	.643
Error(cond)	Sphericity Assumed	4437.978	8	554.747		
	Greenhouse-Geisser	4437.978	8.000	554.747		
	Huynh-Feldt	4437.978	8.000	554.747		
	Lower-bound	4437.978	8.000	554.747		
time	Sphericity Assumed	10224.144	8	1278.018	7.491	.000
	Greenhouse-Geisser	10224.144	2.639	3874.976	7.491	.002
	Huynh-Feldt	10224.144	4.548	2247.964	7.491	.000
	Lower-bound	10224.144	1.000	10224.144	7.491	.026
time * group	Sphericity Assumed	1683.300	8	210.412	1.233	.295
	Greenhouse-Geisser	1683.300	2.639	637.975	1.233	.320
	Huynh-Feldt	1683.300	4.548	370.104	1.233	.314
	Lower-bound	1683.300	1.000	1683.300	1.233	.299
Error(time)	Sphericity Assumed	10918.667	64	170.604		
	Greenhouse-Geisser	10918.667	21.108	517.275		
	Huynh-Feldt	10918.667	36.385	300.083		
	Lower-bound	10918.667	8.000	1364.833		
cond * time	Sphericity Assumed	1863.500	8	232.937	1.205	.311
	Greenhouse-Geisser	1863.500	2.242	831.171	1.205	.327
	Huynh-Feldt	1863.500	3.546	525.461	1.205	.329
	Lower-bound	1863.500	1.000	1863.500	1.205	.304
cond * time * group	Sphericity Assumed	442.344	8	55.293	.286	.968
	Greenhouse-Geisser	442.344	2.242	197.298	.286	.778
	Huynh-Feldt	442.344	3.546	124.730	.286	.865
	Lower-bound	442.344	1.000	442.344	.286	.607
Error(cond*time)	Sphericity Assumed	12375.822	64	193.372		
	Greenhouse-Geisser	12375.822	17.936	689.994		
	Huynh-Feldt	12375.822	28.371	436.209		
	Lower-bound	12375.822	8.000	1546.978		

Fullness

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	224.450	1	224.450	.638	.447
	Greenhouse-Geisser	224.450	1.000	224.450	.638	.447
	Huynh-Feldt	224.450	1.000	224.450	.638	.447
	Lower-bound	224.450	1.000	224.450	.638	.447
cond * group	Sphericity Assumed	238.050	1	238.050	.677	.435
	Greenhouse-Geisser	238.050	1.000	238.050	.677	.435
	Huynh-Feldt	238.050	1.000	238.050	.677	.435
	Lower-bound	238.050	1.000	238.050	.677	.435
Error(cond)	Sphericity Assumed	2814.333	8	351.792		
	Greenhouse-Geisser	2814.333	8.000	351.792		
	Huynh-Feldt	2814.333	8.000	351.792		
	Lower-bound	2814.333	8.000	351.792		
time	Sphericity Assumed	1914.700	8	239.337	1.151	.343
	Greenhouse-Geisser	1914.700	2.146	892.103	1.151	.343
	Huynh-Feldt	1914.700	3.325	575.875	1.151	.350
	Lower-bound	1914.700	1.000	1914.700	1.151	.315
time * group	Sphericity Assumed	1373.611	8	171.701	.826	.583
	Greenhouse-Geisser	1373.611	2.146	639.997	.826	.462
	Huynh-Feldt	1373.611	3.325	413.134	.826	.502
	Lower-bound	1373.611	1.000	1373.611	.826	.390
Error(time)	Sphericity Assumed	13307.800	64	207.934		
	Greenhouse-Geisser	13307.800	17.170	775.051		
	Huynh-Feldt	13307.800	26.599	500.315		
	Lower-bound	13307.800	8.000	1663.475		
cond * time	Sphericity Assumed	1974.700	8	246.838	2.453	.022
	Greenhouse-Geisser	1974.700	3.394	581.746	2.453	.078
	Huynh-Feldt	1974.700	6.936	284.702	2.453	.029
	Lower-bound	1974.700	1.000	1974.700	2.453	.156
cond * time * group	Sphericity Assumed	377.300	8	47.162	.469	.874
	Greenhouse-Geisser	377.300	3.394	111.152	.469	.729
	Huynh-Feldt	377.300	6.936	54.397	.469	.852
	Lower-bound	377.300	1.000	377.300	.469	.513
Error(cond*time)	Sphericity Assumed	6439.667	64	100.620		
	Greenhouse-Geisser	6439.667	27.155	237.141		
	Huynh-Feldt	6439.667	55.488	116.055		
	Lower-bound	6439.667	8.000	804.958		

20 Minute Fullness NSEP versus SED

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	781.250	1	781.250	6.269	.037
	Greenhouse-Geisser	781.250	1.000	781.250	6.269	.037
	Huynh-Feldt	781.250	1.000	781.250	6.269	.037
	Lower-bound	781.250	1.000	781.250	6.269	.037
cond * group	Sphericity Assumed	1.250	1	1.250	.010	.923
	Greenhouse-Geisser	1.250	1.000	1.250	.010	.923
	Huynh-Feldt	1.250	1.000	1.250	.010	.923
	Lower-bound	1.250	1.000	1.250	.010	.923
Error(cond)	Sphericity Assumed	997.000	8	124.625		
	Greenhouse-Geisser	997.000	8.000	124.625		
	Huynh-Feldt	997.000	8.000	124.625		
	Lower-bound	997.000	8.000	124.625		

Days 3, 4, 5, 6 and 7 (Intervention day and follow-up days)

Hunger

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	231.040	1	231.040	3.413	.102
	Greenhouse-Geisser	231.040	1.000	231.040	3.413	.102
	Huynh-Feldt	231.040	1.000	231.040	3.413	.102
	Lower-bound	231.040	1.000	231.040	3.413	.102
cond * group	Sphericity Assumed	19.360	1	19.360	.286	.607
	Greenhouse-Geisser	19.360	1.000	19.360	.286	.607
	Huynh-Feldt	19.360	1.000	19.360	.286	.607
	Lower-bound	19.360	1.000	19.360	.286	.607
Error(cond)	Sphericity Assumed	541.600	8	67.700		
	Greenhouse-Geisser	541.600	8.000	67.700		
	Huynh-Feldt	541.600	8.000	67.700		
	Lower-bound	541.600	8.000	67.700		
day	Sphericity Assumed	337.200	4	84.300	.917	.466
	Greenhouse-Geisser	337.200	2.368	142.382	.917	.432
	Huynh-Feldt	337.200	3.850	87.581	.917	.464
	Lower-bound	337.200	1.000	337.200	.917	.366
day * group	Sphericity Assumed	455.200	4	113.800	1.238	.315
	Greenhouse-Geisser	455.200	2.368	192.207	1.238	.318
	Huynh-Feldt	455.200	3.850	118.230	1.238	.315
	Lower-bound	455.200	1.000	455.200	1.238	.298
Error(day)	Sphericity Assumed	2942.200	32	91.944		
	Greenhouse-Geisser	2942.200	18.946	155.292		
	Huynh-Feldt	2942.200	30.801	95.523		
	Lower-bound	2942.200	8.000	367.775		
cond * day	Sphericity Assumed	189.160	4	47.290	.771	.552
	Greenhouse-Geisser	189.160	2.388	79.200	.771	.497
	Huynh-Feldt	189.160	3.900	48.506	.771	.549
	Lower-bound	189.160	1.000	189.160	.771	.405
cond * day * group	Sphericity Assumed	28.040	4	7.010	.114	.977
	Greenhouse-Geisser	28.040	2.388	11.740	.114	.921
	Huynh-Feldt	28.040	3.900	7.190	.114	.975
	Lower-bound	28.040	1.000	28.040	.114	.744
Error(cond*day)	Sphericity Assumed	1961.800	32	61.306		
	Greenhouse-Geisser	1961.800	19.107	102.674		
	Huynh-Feldt	1961.800	31.198	62.882		
	Lower-bound	1961.800	8.000	245.225		

Fullness

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	60.840	1	60.840	.546	.481
	Greenhouse-Geisser	60.840	1.000	60.840	.546	.481
	Huynh-Feldt	60.840	1.000	60.840	.546	.481
	Lower-bound	60.840	1.000	60.840	.546	.481
cond * group	Sphericity Assumed	25.000	1	25.000	.224	.648
	Greenhouse-Geisser	25.000	1.000	25.000	.224	.648
	Huynh-Feldt	25.000	1.000	25.000	.224	.648
	Lower-bound	25.000	1.000	25.000	.224	.648
Error(cond)	Sphericity Assumed	891.960	8	111.495		
	Greenhouse-Geisser	891.960	8.000	111.495		
	Huynh-Feldt	891.960	8.000	111.495		
	Lower-bound	891.960	8.000	111.495		
day	Sphericity Assumed	156.100	4	39.025	.449	.772
	Greenhouse-Geisser	156.100	2.307	67.669	.449	.672
	Huynh-Feldt	156.100	3.701	42.182	.449	.758
	Lower-bound	156.100	1.000	156.100	.449	.522
day * group	Sphericity Assumed	597.740	4	149.435	1.720	.170
	Greenhouse-Geisser	597.740	2.307	259.118	1.720	.204
	Huynh-Feldt	597.740	3.701	161.524	1.720	.176
	Lower-bound	597.740	1.000	597.740	1.720	.226
Error(day)	Sphericity Assumed	2780.760	32	86.899		
	Greenhouse-Geisser	2780.760	18.455	150.681		
	Huynh-Feldt	2780.760	29.605	93.929		
	Lower-bound	2780.760	8.000	347.595		
cond * day	Sphericity Assumed	302.660	4	75.665	.822	.521
	Greenhouse-Geisser	302.660	2.312	130.926	.822	.471
	Huynh-Feldt	302.660	3.712	81.528	.822	.514
	Lower-bound	302.660	1.000	302.660	.822	.391
cond * day * group	Sphericity Assumed	60.700	4	15.175	.165	.955
	Greenhouse-Geisser	60.700	2.312	26.258	.165	.877
	Huynh-Feldt	60.700	3.712	16.351	.165	.947
	Lower-bound	60.700	1.000	60.700	.165	.695
Error(cond*day)	Sphericity Assumed	2946.840	32	92.089		
	Greenhouse-Geisser	2946.840	18.494	159.344		
	Huynh-Feldt	2946.840	29.699	99.225		
	Lower-bound	2946.840	8.000	368.355		

Prospective Food Consumption

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	121.000	1	121.000	1.136	.318
	Greenhouse-Geisser	121.000	1.000	121.000	1.136	.318
	Huynh-Feldt	121.000	1.000	121.000	1.136	.318
	Lower-bound	121.000	1.000	121.000	1.136	.318
cond * group	Sphericity Assumed	38.440	1	38.440	.361	.565
	Greenhouse-Geisser	38.440	1.000	38.440	.361	.565
	Huynh-Feldt	38.440	1.000	38.440	.361	.565
	Lower-bound	38.440	1.000	38.440	.361	.565
Error(cond)	Sphericity Assumed	852.160	8	106.520		
	Greenhouse-Geisser	852.160	8.000	106.520		
	Huynh-Feldt	852.160	8.000	106.520		
	Lower-bound	852.160	8.000	106.520		
day	Sphericity Assumed	263.940	4	65.985	.957	.444
	Greenhouse-Geisser	263.940	2.371	111.305	.957	.415
	Huynh-Feldt	263.940	3.858	68.421	.957	.442
	Lower-bound	263.940	1.000	263.940	.957	.357
day * group	Sphericity Assumed	367.900	4	91.975	1.334	.279
	Greenhouse-Geisser	367.900	2.371	155.146	1.334	.290
	Huynh-Feldt	367.900	3.858	95.370	1.334	.280
	Lower-bound	367.900	1.000	367.900	1.334	.281
Error(day)	Sphericity Assumed	2205.760	32	68.930		
	Greenhouse-Geisser	2205.760	18.971	116.273		
	Huynh-Feldt	2205.760	30.861	71.475		
	Lower-bound	2205.760	8.000	275.720		
cond * day	Sphericity Assumed	355.700	4	88.925	1.164	.345
	Greenhouse-Geisser	355.700	2.577	138.054	1.164	.342
	Huynh-Feldt	355.700	4.000	88.925	1.164	.345
	Lower-bound	355.700	1.000	355.700	1.164	.312
cond * day * group	Sphericity Assumed	101.260	4	25.315	.331	.855
	Greenhouse-Geisser	101.260	2.577	39.301	.331	.773
	Huynh-Feldt	101.260	4.000	25.315	.331	.855
	Lower-bound	101.260	1.000	101.260	.331	.581
Error(cond*day)	Sphericity Assumed	2445.440	32	76.420		
	Greenhouse-Geisser	2445.440	20.612	118.641		
	Huynh-Feldt	2445.440	32.000	76.420		
	Lower-bound	2445.440	8.000	305.680		

Acylated Ghrelin: NSEP and SED Interventions

Trained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1156.403	1	1156.403	.253	.649
	Greenhouse-Geisser	1156.403	1.000	1156.403	.253	.649
	Huynh-Feldt	1156.403	1.000	1156.403	.253	.649
	Lower-bound	1156.403	1.000	1156.403	.253	.649
Error(cond)	Sphericity Assumed	13698.042	3	4566.014		
	Greenhouse-Geisser	13698.042	3.000	4566.014		
	Huynh-Feldt	13698.042	3.000	4566.014		
	Lower-bound	13698.042	3.000	4566.014		
time	Sphericity Assumed	2544.129	5	508.826	1.128	.388
	Greenhouse-Geisser	2544.129	1.186	2145.159	1.128	.372
	Huynh-Feldt	2544.129	1.513	1681.915	1.128	.378
	Lower-bound	2544.129	1.000	2544.129	1.128	.366
Error(time)	Sphericity Assumed	6768.126	15	451.208		
	Greenhouse-Geisser	6768.126	3.558	1902.249		
	Huynh-Feldt	6768.126	4.538	1491.462		
	Lower-bound	6768.126	3.000	2256.042		
cond * time	Sphericity Assumed	854.152	5	170.830	.510	.764
	Greenhouse-Geisser	854.152	1.677	509.253	.510	.599
	Huynh-Feldt	854.152	3.560	239.925	.510	.712
	Lower-bound	854.152	1.000	854.152	.510	.527
Error(cond*time)	Sphericity Assumed	5023.053	15	334.870		
	Greenhouse-Geisser	5023.053	5.032	998.264		
	Huynh-Feldt	5023.053	10.680	470.312		
	Lower-bound	5023.053	3.000	1674.351		

Untrained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	45.141	1	45.141	.369	.605
	Greenhouse-Geisser	45.141	1.000	45.141	.369	.605
	Huynh-Feldt	45.141	1.000	45.141	.369	.605
	Lower-bound	45.141	1.000	45.141	.369	.605
Error(cond)	Sphericity Assumed	244.739	2	122.369		
	Greenhouse-Geisser	244.739	2.000	122.369		
	Huynh-Feldt	244.739	2.000	122.369		
	Lower-bound	244.739	2.000	122.369		
time	Sphericity Assumed	6044.702	4	1511.176	14.264	.001
	Greenhouse-Geisser	6044.702	1.957	3089.019	14.264	.016
	Huynh-Feldt	6044.702	4.000	1511.176	14.264	.001
	Lower-bound	6044.702	1.000	6044.702	14.264	.064
Error(time)	Sphericity Assumed	847.536	8	105.942		
	Greenhouse-Geisser	847.536	3.914	216.558		
	Huynh-Feldt	847.536	8.000	105.942		
	Lower-bound	847.536	2.000	423.768		
cond * time	Sphericity Assumed	3403.949	4	850.987	1.434	.307
	Greenhouse-Geisser	3403.949	1.106	3077.711	1.434	.353
	Huynh-Feldt	3403.949	1.474	2308.900	1.434	.348
	Lower-bound	3403.949	1.000	3403.949	1.434	.354
Error(cond*time)	Sphericity Assumed	4747.521	8	593.440		
	Greenhouse-Geisser	4747.521	2.212	2146.257		
	Huynh-Feldt	4747.521	2.949	1610.123		
	Lower-bound	4747.521	2.000	2373.761		

Whole Group

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	180.161	1	180.161	.099	.764
	Greenhouse-Geisser	180.161	1.000	180.161	.099	.764
	Huynh-Feldt	180.161	1.000	180.161	.099	.764
	Lower-bound	180.161	1.000	180.161	.099	.764
Error(cond)	Sphericity Assumed	10950.128	6	1825.021		
	Greenhouse-Geisser	10950.128	6.000	1825.021		
	Huynh-Feldt	10950.128	6.000	1825.021		
	Lower-bound	10950.128	6.000	1825.021		
time	Sphericity Assumed	6285.198	4	1571.300	3.947	.013
	Greenhouse-Geisser	6285.198	1.836	3423.452	3.947	.054
	Huynh-Feldt	6285.198	2.606	2411.844	3.947	.032
	Lower-bound	6285.198	1.000	6285.198	3.947	.094
Error(time)	Sphericity Assumed	9553.732	24	398.072		
	Greenhouse-Geisser	9553.732	11.016	867.296		
	Huynh-Feldt	9553.732	15.636	611.015		
	Lower-bound	9553.732	6.000	1592.289		
cond * time	Sphericity Assumed	3020.764	4	755.191	1.932	.138
	Greenhouse-Geisser	3020.764	2.126	1421.142	1.932	.184
	Huynh-Feldt	3020.764	3.324	908.732	1.932	.153
	Lower-bound	3020.764	1.000	3020.764	1.932	.214
Error(cond*time)	Sphericity Assumed	9380.322	24	390.847		
	Greenhouse-Geisser	9380.322	12.754	735.508		
	Huynh-Feldt	9380.322	19.945	470.312		
	Lower-bound	9380.322	6.000	1563.387		

Insulin: NSEP and SED Interventions

Trained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	25.244	1	25.244	1.588	.297
	Greenhouse-Geisser	25.244	1.000	25.244	1.588	.297
	Huynh-Feldt	25.244	1.000	25.244	1.588	.297
	Lower-bound	25.244	1.000	25.244	1.588	.297
Error(cond)	Sphericity Assumed	47.694	3	15.898		
	Greenhouse-Geisser	47.694	3.000	15.898		
	Huynh-Feldt	47.694	3.000	15.898		
	Lower-bound	47.694	3.000	15.898		
time	Sphericity Assumed	275.146	5	55.029	8.834	.000
	Greenhouse-Geisser	275.146	1.604	171.582	8.834	.027
	Huynh-Feldt	275.146	3.161	87.039	8.834	.004
	Lower-bound	275.146	1.000	275.146	8.834	.059
Error(time)	Sphericity Assumed	93.441	15	6.229		
	Greenhouse-Geisser	93.441	4.811	19.423		
	Huynh-Feldt	93.441	9.483	9.853		
	Lower-bound	93.441	3.000	31.147		
cond * time	Sphericity Assumed	69.886	5	13.977	.528	.752
	Greenhouse-Geisser	69.886	1.289	54.201	.528	.554
	Huynh-Feldt	69.886	1.846	37.861	.528	.603
	Lower-bound	69.886	1.000	69.886	.528	.520
Error(cond*time)	Sphericity Assumed	396.954	15	26.464		
	Greenhouse-Geisser	396.954	3.868	102.621		
	Huynh-Feldt	396.954	5.538	71.684		
	Lower-bound	396.954	3.000	132.318		

Untrained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	40.588	1	40.588	2.052	.288
	Greenhouse-Geisser	40.588	1.000	40.588	2.052	.288
	Huynh-Feldt	40.588	1.000	40.588	2.052	.288
	Lower-bound	40.588	1.000	40.588	2.052	.288
Error(cond)	Sphericity Assumed	39.563	2	19.781		
	Greenhouse-Geisser	39.563	2.000	19.781		
	Huynh-Feldt	39.563	2.000	19.781		
	Lower-bound	39.563	2.000	19.781		
time	Sphericity Assumed	134.687	5	26.937	1.177	.385
	Greenhouse-Geisser	134.687	1.154	116.733	1.177	.393
	Huynh-Feldt	134.687	1.727	77.989	1.177	.396
	Lower-bound	134.687	1.000	134.687	1.177	.391
Error(time)	Sphericity Assumed	228.783	10	22.878		
	Greenhouse-Geisser	228.783	2.308	99.143		
	Huynh-Feldt	228.783	3.454	66.237		
	Lower-bound	228.783	2.000	114.391		
cond * time	Sphericity Assumed	94.016	5	18.803	1.544	.261
	Greenhouse-Geisser	94.016	1.760	53.425	1.544	.324
	Huynh-Feldt	94.016	5.000	18.803	1.544	.261
	Lower-bound	94.016	1.000	94.016	1.544	.340
Error(cond*time)	Sphericity Assumed	121.819	10	12.182		
	Greenhouse-Geisser	121.819	3.520	34.612		
	Huynh-Feldt	121.819	10.000	12.182		
	Lower-bound	121.819	2.000	60.909		

Whole Group

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	63.500	1	63.500	4.253	.085
	Greenhouse-Geisser	63.500	1.000	63.500	4.253	.085
	Huynh-Feldt	63.500	1.000	63.500	4.253	.085
	Lower-bound	63.500	1.000	63.500	4.253	.085
Error(cond)	Sphericity Assumed	89.588	6	14.931		
	Greenhouse-Geisser	89.588	6.000	14.931		
	Huynh-Feldt	89.588	6.000	14.931		
	Lower-bound	89.588	6.000	14.931		
time	Sphericity Assumed	390.907	5	78.181	6.875	.000
	Greenhouse-Geisser	390.907	1.530	255.454	6.875	.019
	Huynh-Feldt	390.907	1.949	200.564	6.875	.011
	Lower-bound	390.907	1.000	390.907	6.875	.039
Error(time)	Sphericity Assumed	341.149	30	11.372		
	Greenhouse-Geisser	341.149	9.181	37.156		
	Huynh-Feldt	341.149	11.694	29.172		
	Lower-bound	341.149	6.000	56.858		
cond * time	Sphericity Assumed	110.556	5	22.111	1.159	.352
	Greenhouse-Geisser	110.556	1.779	62.158	1.159	.344
	Huynh-Feldt	110.556	2.476	44.659	1.159	.350
	Lower-bound	110.556	1.000	110.556	1.159	.323
Error(cond*time)	Sphericity Assumed	572.119	30	19.071		
	Greenhouse-Geisser	572.119	10.672	53.611		
	Huynh-Feldt	572.119	14.854	38.517		
	Lower-bound	572.119	6.000	95.353		

Glucose: NSEP and SED Interventions

Trained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	3.699	1	3.699	1.544	.302
	Greenhouse-Geisser	3.699	1.000	3.699	1.544	.302
	Huynh-Feldt	3.699	1.000	3.699	1.544	.302
	Lower-bound	3.699	1.000	3.699	1.544	.302
Error(cond)	Sphericity Assumed	7.186	3	2.395		
	Greenhouse-Geisser	7.186	3.000	2.395		
	Huynh-Feldt	7.186	3.000	2.395		
	Lower-bound	7.186	3.000	2.395		
time	Sphericity Assumed	.254	5	.051	.313	.897
	Greenhouse-Geisser	.254	1.486	.171	.313	.687
	Huynh-Feldt	.254	2.604	.097	.313	.791
	Lower-bound	.254	1.000	.254	.313	.615
Error(time)	Sphericity Assumed	2.431	15	.162		
	Greenhouse-Geisser	2.431	4.457	.546		
	Huynh-Feldt	2.431	7.811	.311		
	Lower-bound	2.431	3.000	.810		
cond * time	Sphericity Assumed	1.810	5	.362	3.400	.030
	Greenhouse-Geisser	1.810	1.740	1.040	3.400	.116
	Huynh-Feldt	1.810	3.935	.460	3.400	.046
	Lower-bound	1.810	1.000	1.810	3.400	.162
Error(cond*time)	Sphericity Assumed	1.597	15	.106		
	Greenhouse-Geisser	1.597	5.219	.306		
	Huynh-Feldt	1.597	11.805	.135		
	Lower-bound	1.597	3.000	.532		

Untrained

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	.032	1	.032	.178	.714
	Greenhouse-Geisser	.032	1.000	.032	.178	.714
	Huynh-Feldt	.032	1.000	.032	.178	.714
	Lower-bound	.032	1.000	.032	.178	.714
Error(cond)	Sphericity Assumed	.355	2	.177		
	Greenhouse-Geisser	.355	2.000	.177		
	Huynh-Feldt	.355	2.000	.177		
	Lower-bound	.355	2.000	.177		
time	Sphericity Assumed	.849	5	.170	1.782	.204
	Greenhouse-Geisser	.849	1.505	.564	1.782	.296
	Huynh-Feldt	.849	5.000	.170	1.782	.204
	Lower-bound	.849	1.000	.849	1.782	.314
Error(time)	Sphericity Assumed	.952	10	.095		
	Greenhouse-Geisser	.952	3.010	.316		
	Huynh-Feldt	.952	10.000	.095		
	Lower-bound	.952	2.000	.476		
cond * time	Sphericity Assumed	.933	5	.187	3.403	.047
	Greenhouse-Geisser	.933	1.194	.781	3.403	.190
	Huynh-Feldt	.933	1.964	.475	3.403	.139
	Lower-bound	.933	1.000	.933	3.403	.206
Error(cond*time)	Sphericity Assumed	.548	10	.055		
	Greenhouse-Geisser	.548	2.388	.230		
	Huynh-Feldt	.548	3.927	.140		
	Lower-bound	.548	2.000	.274		

Whole Group

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
cond	Sphericity Assumed	1.789	1	1.789	1.132	.328
	Greenhouse-Geisser	1.789	1.000	1.789	1.132	.328
	Huynh-Feldt	1.789	1.000	1.789	1.132	.328
	Lower-bound	1.789	1.000	1.789	1.132	.328
Error(cond)	Sphericity Assumed	9.482	6	1.580		
	Greenhouse-Geisser	9.482	6.000	1.580		
	Huynh-Feldt	9.482	6.000	1.580		
	Lower-bound	9.482	6.000	1.580		
time	Sphericity Assumed	.322	5	.064	.465	.799
	Greenhouse-Geisser	.322	2.137	.151	.465	.651
	Huynh-Feldt	.322	3.355	.096	.465	.730
	Lower-bound	.322	1.000	.322	.465	.521
Error(time)	Sphericity Assumed	4.163	30	.139		
	Greenhouse-Geisser	4.163	12.823	.325		
	Huynh-Feldt	4.163	20.131	.207		
	Lower-bound	4.163	6.000	.694		
cond * time	Sphericity Assumed	2.579	5	.516	6.699	.000
	Greenhouse-Geisser	2.579	2.248	1.147	6.699	.008
	Huynh-Feldt	2.579	3.662	.704	6.699	.001
	Lower-bound	2.579	1.000	2.579	6.699	.041
Error(cond*time)	Sphericity Assumed	2.310	30	.077		
	Greenhouse-Geisser	2.310	13.489	.171		
	Huynh-Feldt	2.310	21.970	.105		
	Lower-bound	2.310	6.000	.385		

Exercise Intensity

ANOVA

pertVO2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	96.100	1	96.100	1.378	.274
Within Groups	558.000	8	69.750		
Total	654.100	9			

Heart Rate

ANOVA

HR1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1562.500	1	1562.500	5.959	.040
Within Groups	2097.600	8	262.200		
Total	3660.100	9			

APPENDIX C

Example of a food diary

My very own.........

FOOD DIARY

Chef Number: ______________

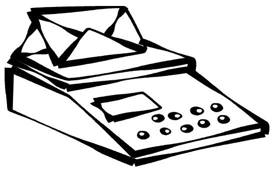
If you have any queries please do not hesitate to contact myself, Penny Rumbold on (0191) 243 7018 or by

Email: penny.rumbold@unn.ac.uk

FOOD DIARY – Become the next best thing to JAMIE OLIVER – a professional chef!

Hello! Are you ready to keep your very own food diary? It means you must record EVERYTHING you EAT and DRINK! You need to do this for the full week (Monday-Friday) when you have exercised and even when you haven't.

To make it easier for you I have included a set of instructions for you to follow. And if you are really good you can even keep food wrappers or drinks bottles and hand them in with your diary when you have finished!



Here we go……..

- **Weighing your food:**

When you wake up in the morning and your sitting down to your breakfast…….make sure you weigh whatever you are going to eat first, with your own set of scales that I have given you. E.g. 2 slices of toast = 20g, if you leave some weigh the left over's which may be 5g, therefore you have eaten 15g. If you had butter and jam – write down the brands and approximately how much you put on your toast = 1 teaspoon of each maybe! Do the same for your packed lunch and your dinner. If you have school dinners I will be at school to help you – so don't worry.

ONLY RIGHT DOWN WHAT YOU EAT or DRINK….. and don't forget about snacks! If you have a mars bars at break time keep the wrapper and attach it to the diary. Write down the time you ate it in your diary!

- **How was the food cooked?**

So, now you have done the hard part……the fun bit…..

Every time you eat something you MUST right down how it was cooked (your mum and dad) can help you with this if you're not sure!

1. BOILED – was it boiled in a pan with water?
2. FRIED – was it cooked in the frying pan with oil?
3. ROASTED – was it roasted in the oven?
4. GRILLED – was it put underneath the grill and cooked?

(Maybe you could help your parents cook to see exactly how things are cooked for yourself!)

Keep checking the list of foods, so you don't forget to record everything, AND don't forget about those SNACKS….. chocolate, crisps, fizzy drinks etc… If your parents cook anything ask them for the RECIPE and write this in the diary too!!

CHECKLIST

Food/Drink	Description & Preparation	Amount (remember you can write the g, ml or kg values if you have them!)
Bacon	Lean or streaky? Fried or grilled rashers?	Number
Baked beans	Normal or reduced sugar/salt?	Tablespoons, tin size or Picture?
Beef burger/Hamburger	Home-made, packet or take-away? Fried, micro-waved or grilled? With bread roll?	Number
Biscuits	Plain, chocolate, sweet, crisp bread, cheese, wafer, home-made? What brand?	Number
Bread	Wholemeal, granary, white, multigrain? Currant, fruit, malt? Large or small loaf? Thick, medium or thin slices? Brand?	Number of slices
Bread rolls	Wholemeal, granary, white? Size? Crusty or soft? Brand? If with filling remember to record it!	Number of rolls
Breakfast cereal	What sort? Cornflakes, Weetabix etc.. What brand?	Number, tablespoons or Picture?
Bun	What sort: Iced, currant, sweet or plain? Large or small? Brand?	Number
Butter	Ordinary or lo fat spread? Brand?	Spread: thickly, average or thinly
Cake - small and large	What sort: Cream, iced, chocolate etc..? Brand?	Number, slices or Picture?
Cheese	What sort: hard, soft, spread, cream, lo-fat, mature, mild? Brand?	Tablespoons or Picture?
Chips	Frozen, oven, microwave, crinkle-cut, chip-shop, MacDonald's etc? How cooked? In what oil? Brand?	Picture?
Chocolate	What sort: Milk, white, plain? Name and Brand?	Number or weight of bar
Chops	What sort; Lamb or Pork? Lean or fatty? Large or small? Fried, grilled, baked?	Number
Coffee	Include milk & sugar! Skimmed, semi, whole milk?	How much milk/sugar?
Cooking oil	Type, Brand?	How many teaspoons/tablespoons?

Cream	Half, whipping, single, double, clotted, lo-fat, fresh or substitute?	How many tablespoons?
Crisps	Brand name? Normal, lo-fat or lo-salt?	Packet weight
Egg	How was it cooked: boiled, fried, scrambled, poached, omelette etc.	Number and size
Fish	What sort; Fried, boiled, grilled, poached, micro waved? Pickled, smoked, salted? Batter, breadcrumbs? Tinned with oil or tomato sauce? Size? Brand where possible?	How much? Or picture?
Fish fingers/cakes	What sort; large, medium or small? Fried or grilled? Brand?	Number
Fruit - fresh	What type (eg banana, apple, and orange)? Brand (eg Bramley, Golden Delicious)?	Number
Fruit - canned/stewed	In fruit juice or syrup? Type of fruit? With or without sugar?	Tablespoons or tin size
Fruit - juice	What sort; sweetened or unsweetened? Brand?	Glasses or cups, small, medium or large
Gravy	Thick or thin? Instant, packet or homemade?	Tablespoons
Honey	Brand? Clear?	Teaspoons
Ice-cream	Dairy or non-dairy? Flavour, variety? Brand?	Tablespoons
Jam	Brand? Normal or lo-sugar?	Teaspoons
Kidney	Fried or stewed? Pig, lamb or ox?	Picture??
Liver	Fried or stewed? Pig, lamb or ox?	Picture??
Margarine	Soft, hard? Polyunsaturated, lo-fat, very lo-fat? Brand?	Spread thickly, average or thinly
Marmalade	Brand? Normal or lo-sugar?	Teaspoons? Spread thickly, average or thinly
Mayonnaise	Brand? Normal or lo-fat?	Teaspoons
Meat	What sort; lean or fatty? Fried, grilled, roast, BBQ, micro waved etc..? Any gravy? If so see GRAVY on checklist!	Slices, helping or Pictures??
Milk	Full cream, semi-skimmed, skimmed? Sterilized, UHT, flavoured, powdered, Soya?	Pints, glasses or cups

Mince	Beef or Lamb? On its own, with vegetables, gravy (see VEGETABLES and GRAVY on checklist)? Fatty or lean? Brand?	Tablespoons or Picture?
Pasta, Spaghetti	Canned, fresh or boiled? White or wholemeal? In sauce (see SAUCE on checklist)? Brand?	Tablespoons or Picture?
Pie, Pasty, Pastry	What sort: meat, vegetable, fruit etc..? Individual or a slice? What type of pastry? Brand?	Number or Picture?
Peanuts	Dry roasted or ordinary salted? Brand?	Packet weight
Porridge	How made: with all milk, ↔milk+↔water or cream? Type of milk (see MILK on checklist)? With sugar or honey? Brand?	Small, medium or large bowl
Potatoes	Baked, boiled (with or without skin?), mashed, creamed, fried, chips (see CHIPS on checklist), roast, instant? With butter, margarine etc (see BUTTER or MARGARINE on checklist)?	Tablespoons or Pictures??
Pudding	What sort and brand? Is it jelly, mousse, milk pudding, sponge etc? If with cream, see CREAM on checklist.	Tablespoons, slices or Pictures???
Rice	Brown or white? Boiled or fried? Brand? If rice pudding see PUDDING on checklist.	Tablespoons or Picture?
Salad	What ingredients? If with dressing, what type (eg. Oil, vinegar, mayonnaise, salad cream etc..)?	Tablespoons, slices or Picture?
Sandwiches and rolls	See checklist for BREAD, ROLL, BUN, BUTTER, and MARGARINE. Remember to include fillings.	
Sauce - hot	What sort: savoury or sweet? Thick or thin? Recipe or ingredients if possible? Brand?	Tablespoons or Picture?
Sauce - cold	What sort: eg. Tomato ketchup, brown sauce, soy sauce, salad cream? Brand?	Tablespoons or Picture?
Sausages	Pork, beef, pork & beef? Large or small? How cooked (grilled, fried)? Brand?	Number
Sausage rolls	Large or small? Type of pastry? Brand?	Number

Scones	With currants, sweet or plain, cheese, wholemeal? See checklist if with BUTTER, MARGERINE, JAM etc.	Number
Snacks - in packet	What sort: eg. Cheese straws, Twiglets, pretzels, if mini-biscuits, see BISCUITS on checklist.	Packet weight
Soft drinks	What type; Squash, diluted? Fizzy drinks? Normal, lo-calorie or sugar free? Brand?	Glasses (small, medium, large), or cans
Soup	What sort; Canned packet, instant, and fresh, home-made? Brand?	Tablespoons, bowl or mug
Soya/Quorn	TVP, mince, burgers or tofu?	Number or Pictures??
Spreads	What type? Brand? If in sandwich see SANDWICH in checklist.	½ or ¼ teaspoons; Spread thickly, average or thinly
Sugar	White, brown or Demerara?	Heaped or level teaspoons, how many?
Sweets	What sort? Eg. Toffees, boiled sweets, lollipops, bubble/chewing gum (sugar-free?) etc. Brand?	Number or packet size
Tea	Include milk & sugar!	How much milk/sugar?
Vegetables	What types? Eg. Carrots, broccoli, peas, tomatoes etc. Fresh, frozen or canned How cooked (boiled, fried, grilled, and roasted)? See checklist if with BUTTER, MARGERINE or SAUCE.	Tablespoons or Pictures???
Water	If bottled, does it have added sugar?	Glasses or bottle size
Yoghurt, Fromage frais	What sort: eg. Fruit, toffee, chocolate, natural, Greek, creamy or plain? Brand?	Carton weight/size, or tablespoons/

DAY & DATE ______________________________

TIME	DESCRIPTION OF FOOD	HOW COOKED/PREPARED	WEIGHT BEFORE (g)	WEIGHT AFTER (g)

TIME	DESCRIPTION OF FOOD	HOW COOKED/PREPARED	WEIGHT BEFORE (g)	WEIGHT AFTER (g)

SNACKS

Write down any snacks throughout the day and the time you EAT it or DRINK it: (remember you can keep your food labels too!)

TIME	FOOD/DRINK	BRAND	AMOUNT/SIZE

RECIPES

This is the part that will make you stand out from your friends in becoming a chef. If your parents or you cook anything (homemade) write down the recipe or even photocopy it and attach it to this page! Remember though when you write down what you have eaten you still have to weigh the amount you eat – because obviously you won't have eaten the whole lot yourself!!!!

APPENDIX D

Example of a VAS

SATURDAY—Woken Up

Time completed ____________________

1. *How* ***hungry*** *do you feel now?*

Very hungry — Not at all hungry

2. *How* ***much*** *would you like to eat now?*

A lot — Nothing at all

3. *How* ***full*** *do you feel now?*

Very full — Not full at all

4. *How* ***alert*** *do you feel now?*

Very alert — Not at all

5. *How* ***active*** *do you feel now?*

Very active — Not at all

6. *How* ***tired*** *do you feel now?*

Very tired — Not tired at all

7. *How* ***happy*** *do you feel now?*

Very happy — Not al all

8. *How* ***bored*** *do you feel now?*

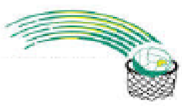
Very bored — Not at all

9. *How* ***relaxed*** *do you feel now?*

Very relaxed — Not at all

10. *What is your* ***overall mood*** *now?*

Good — Bad



APPENDIX E

Example of a retrospective questionnaire

Retrospective Questionnaire Participant:________

1. **Which parts of the study did you find most enjoyable (please circle as many as you like):**

Fitness test with mask on Completing food diaries (weighing food)

Completing VAS diaries Bod Pod Resting under the hood

Remaining inactive Wearing heart rate and activity monitors

Blood taking Netball exercise

Any other parts not stated above ______________________________

2. **Which parts of the study did you find least enjoyable (please circle as many as you like):**

Fitness test with mask on Completing food diaries (weighing food)

Completing VAS diaries Bod Pod Resting under the hood

Remaining inactive Wearing heart rate and activity monitors

Blood taking Netball exercise

Any other parts not stated above ______________________________

3. **Why did you find these least enjoyable?**

____

____

____

4. **Did the NSEP feel like you were playing netball? If yes, why? If no, why?**

YES, why?

____

____

NO,why?____

5. **Did you find it hard to remain inactive during the testing weeks?**

____

6. **Do you think you altered what foods and drinks you consumed because of the diary you were asked to complete?**

____

7. **Did you enjoy taking part in the study?**

____

8. **Would you consider doing a similar study again?**

____

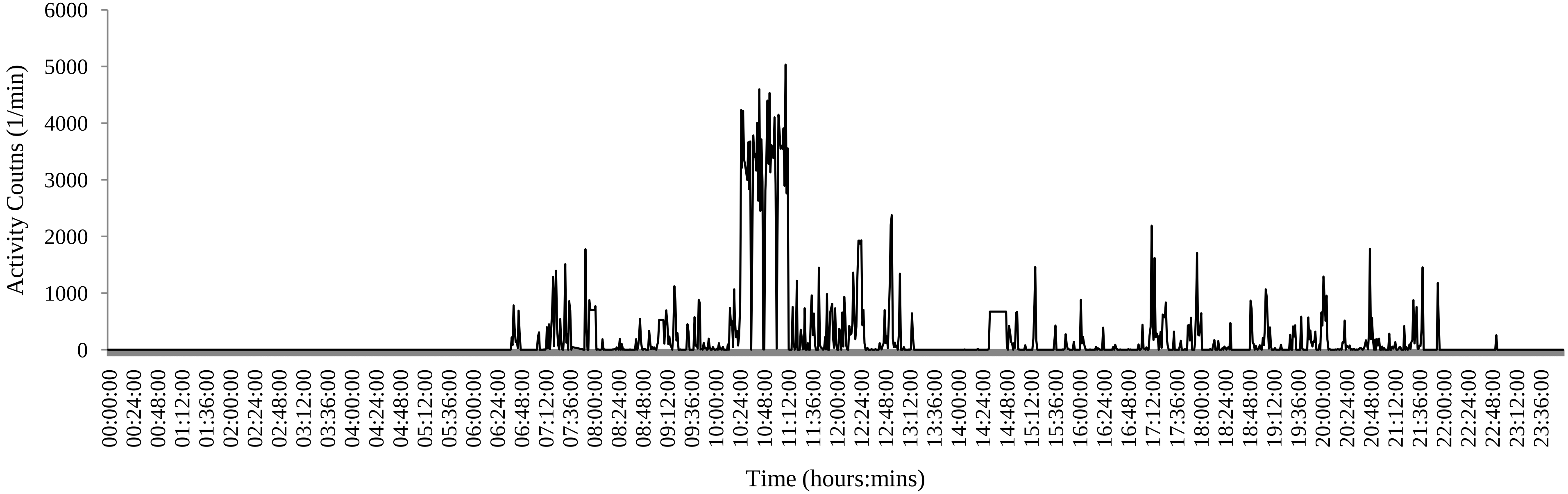
9. **What were your overall thoughts about what you were asked to do?**

____

____

APPENDIX F

Example of accelerometer data



Accelerometer counts for day 3 in the NSEP treatment week for one participant. The elevated counts indicate the period when the NSEP was being completed.

APPENDIX G

Details of the acylated ghrelin assay technique

HUMAN ACYLATED GHRELIN ENZYME IMMUNOASSAY INSTRUCTIONS

Equipment:

Plate reader
Plate shaker
Plate washer
Magnetic stirrer + magnet
Glassware (measuring cylinders, beakers with lids)
100-5000 μL automatic pipette
50-1000 μL automatic pipette
10-300 μL automatic pipette
Pipette tips
Distilled water
Silver foil (to cover the Ellman's reagent on day 2)
Try and keep the room temperature constant on each assay day
Adhesive film for covering the plate

Day 1

1. Take out plasma samples and ghrelin kit and allow them to thaw to room temperature.

2. Label Eppendorf tubes for plasma sample dilution and for standards i.e. eight tubes labelled S1 to S8.

3. Draw out the plate template indicating which standards/samples are in each well using the sheet provided. Label the rows on the plate and which is the top in case any fall out of the case.

4. Make up the **EIA buffer**. Reconstitute one vial with 50 mL of distilled water. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

6. Make up the **wash buffer** in large beaker. Dilute 1 mL of concentrated wash buffer to 400 mL with distilled water (i.e. 1 mL of concentrated wash buffer and 399 mL of distilled water). Add 200 μL of tween 20 – <u>**use a 1000 μL (blue tip) pipette**</u> for the tween.

   Ensure there is enough wash buffer for the assay i.e. 300 μL per well x 96 wells x 15 washes over two days = 432,000 μL or 432 mL. Thus, best to add **1.5 mL** concentrated wash buffer to **598.5 mL** of distilled water making 600 mL of wash buffer. Add **300** μL of tween. **N.B.** Tween is difficult to dispense so get as near to 300 μL as possible. Mix contents using magnetic stirrer.

7. Make up the **stock standard**. Reconstitute one vial with 1 mL of distilled water. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

8. Make up the **quality control**. Reconstitute one vial with 1 mL of distilled water. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

9. Add 200 μL of EIA buffer to labelled Eppendorf tubes (can multi-d on 1000μL pipette). Add 50 μL of sample to labelled Eppendorf tubes i.e. 1 in 5 dilution. This provides enough diluted sample for 2 x 100 μL per Eppendorf tube if measuring samples in duplicate. Ensure that plasma samples are mixed thoroughly prior to diluting with EIA. Use a vortex mixer if available or invert and tap the bottom.

10. Make up additional standards. Dispense 900 μL of stock standard (250 pg/mL) into Eppendorf S1. Dispense 500 μL of EIA buffer into each of the other Eppendorf tubes (S2 to S8). Pipette

500 μL of stock standard (250 pg/mL) from S1 into S2 and mix. Take 500 μL from S2 (125 pg/mL), pipette this into S3 and mix. Continue on with this until all eight standards are complete: S1 = 250, S2 = 125, S3 = 62.5, S4 = 31.3, S5 = 15.6, S6 = 7.81, S7 = 3.91 and S8 = 1.96 pg/mL.

11. Wash the plate with the wash buffer 5 x 300 μL per well. Blot plate on completion. Wells can fall out but this can be avoided by gripping the plate in the middle either side.

12. Mix eppendorfs of EIA and sample whilst plate is washing.

13. Make up the **anti-acylated ghrelin-AChE tracer**. Reconstitute one vial with 10 mL of **EIA buffer**. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

14. Dispensing **-** Leave blank wells empty. No need to include (non-specific binding) wells. Dispense **100 μL of each standard** in duplicate. Dispense **100 μL of the quality control** in duplicate. Dispense **100 μL of each sample** in duplicate or single depending on number of wells available. Do a **CV %** on at least one of the wells.

15. Add tracer – 100 μL into every well **except the blank wells (can** multi D on 1000μL pipette – 10 x 100 μL)

16. Cover the plate with adhesive film. Place the plate in a foil sleeve. Incubate the plate for 20 hours at +4^{o}C in a refrigerator.

17. Put wash buffer into beaker and refrigerate. Put ghrelin kit and all bottles etc in fridge. Can dispose of EIA buffer.

Day 2

1. Take the plate out of the refrigerator, **tip out the contents** and blot on paper.

2. Wash the plate with the wash buffer 5 x 300 μL per well. **Eject the plate** from the plate washer once the 5th wash has been added i.e. before the plate has been aspirated.

3. Cover the plate with adhesive film and shake the plate on the orbital shaker (with the liquid in the wells) for 5 minutes at 300 rpm.

4. Invert the plate to remove the wash buffer and then re-wash the plate with wash buffer 5 x 300 μL per well. Blot the plate on paper.

5. Make up the **Ellman's reagent** 5 minutes before it is required into a bottle rather than beaker. Reconstitute with **49 mL of distilled water** and **1 mL of concentrated wash buffer**. Mix thoroughly and wrap the glass bottle in foil to keep dark (or use a brown bottle) as light sensitive. Dimmed lights.

6. Add 200 μL of Ellman's reagent to each well **including the blank** (multi D on 1000μL pipette)

7. Cover the plate with adhesive film and place it in a foil sleeve. Incubate the plate for 30 minutes in the dark at room temperature.

5. Read the plate at 405 nm. The data should be plotted using a Spline curve.

6. The regression equation **catox(avg(od))** will need to be added to "Advanced calculations" – "Evaluations" – "Long Result"

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