Abstract

Purpose: To examine the impact of high protein intake on whole body composition changes in total body mass, lean body mass and fat mass as well as exercise performance following a short-term hypoenergetic diet in active females. **Methods:** In a parallel design, 18 healthy active females were prescribed 100% of their habitual energy intake for one week. Participants were then assigned to a hypoenergetic diet (60% of habitual energy intake) consisting of either a normal protein diet (CON, 15% protein total energy intake, n = 9) or a high protein diet (HP, 35% protein total energy intake, n = 9) for a 2-week period. Macronutrient composition during the hypoenergetic diet was set at 15% PRO, 50% CHO, 35% FAT for CON and 35% PRO, 50% CHO, FAT 15% in HP. Total body mass, lean body mass, fat mass and exercise performance (anaerobic power, isokinetic strength, speed and anaerobic endurance) were assessed at the end of the 100% habitual prescribed diet and upon completion of 2 weeks hypoenergetic diet intervention. **Results:** No significant differences in any measurement of body composition were detected between CON and HP. Total body mass, (CON: -1.1 ± 1.1kg; HP: -1.0 ± 0.7kg, p=0.85) lean body mass (CON: –0.4kg ± 1.1kg; HP: -0.1kg ± 0.7kg, p=0.55) and fat mass (CON: -0.7± 0.6kg; HP -0.9 ± 0.4kg, p=0.43) reductions were observed. Further mechanistic-based inferential statistical analysis observed a potential substantially positive effect (52%) of attenuating lean body mass with higher protein. Exercise performance was maintained throughout the duration of the study independent of dietary protein intake. **Conclusion:** It is concluded that a high protein intake (35%) compared to a normal protein intake (15%) during energy restriction does not significantly alter body composition or exercise performance in active females during a short term hypoenergetic diet.

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± SEM. CON, control. HP, high protein. Secs, seconds, N= Nanometres, W= Watts, M= Metres.

Table 6: Pre and Post diet intervention exercise performance test scores in both the HP and CON group. Values are expressed as mean ± SEM. CON, control. HP, high protein. Secs= seconds, N=Nanometres, W= Watts, M=Metres. No significant ($p \geq 0.05$) treatment×time interactions were found in any of the exercise performance tests from pre to post intervention diet testing.

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List of Abbreviations

CHO	Carbohydrate
CON	Control
ER	Energy Restriction
FM	Fat Mass
FP	Follicular phase
$g \cdot kg^{-1}$ BM$\cdot d^{-1}$	Grams per kilogram of body mass per day
HP	High Protein
kg	Kilogram
LBM	Lean Body Mass
LP	Luteal Phase
m	Metres
MPS	Muscle Protein Synthesis
Ng/mL	Nanogram/milliliter
nm	Nanometre
PRO	Protein
SD	Standard Deviation
SEM	Standard error of the mean
secs	Seconds
TBM	Total Body Mass
W	Watts

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Authors Declaration

I declare that the work contained within this thesis is of my own production. The work within the thesis has been submitted for and only the award of MPhil.

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Chapter 1: Introduction

1.1Relevance to athletes

Weight loss is an important consideration for many athletes competing at the highest level for several reasons ranging from aesthetic reasons to performance benefits. Weight loss strategies are commonly undertaken by athletes involved in boxing, wrestling and judo to meet required weight categories, aesthetic based activities such as body building and dance also commonly use weight loss strategies to reduce fat mass (FM) prior to competition. In addition, sports that necessitate a high power:mass ratio such as rowers, sprinters and cyclists will employ weight loss strategies (18,28,30). Weight loss is typically attained through energy restriction to maintain or attain a better power to mass ratio. However, weight loss resulting from energy restriction often results in a reduction of lean body mass and can consequently have a negative effect on performance in athletes (11, 28). Therefore, to minimise the negative effect of performance, athletes should aim to preserve lean body mass (LBM) during energy restriction (ER) by implementing a “high quality” weight loss strategy. This weight loss strategy of reducing total body mass (TBM) by decreasing fat mass (FM) and maintaining LBM could subsequently translate into a competitive advantage (10, 23, 21, 29).

1.2 Dietary weight loss interventions for the preservation of lean muscle mass.

A growing body of evidence suggests that increasing the protein content of the diet during ER plays an important role in preserving LBM during weight loss (19, 8, 30). Indeed, increasing the protein content of the diet with exercise training (12, 13, 10) has been shown to elicit a greater loss of total body mass (TBM) and fat mass (FM) compared with a diet consisting of a habitual protein intake. While it is well established that an increased protein hypoenergetic diet can ameliorate the loss of LBM during weight loss in obese and overweight individuals (12, 13, 14), there is little

evidence of the same results in the athletic population, in particular female athletes. Leaner athletes have a large proportion of LBM and therefore are potentially vulnerable to LBM loss during ER. The need for increased protein is of particular interest to the athletic population when trying to modify body composition and sustain performance at the same time. The recommended daily allowance of protein in the general population during energy balance had been set at 0.8 g·kg^{-1} BM however, the amount of protein required during periods of ER is not fully understood. It has been reported that during ER, dietary protein consumption may need to be increased to aid positive body composition changes (5, 7, 17). Dietary interventions promoting an increase in protein intakes beyond the RDA have been researched, ranging from 1.2 to 2.7 g·kg^{-1} BM· across different populations and time phases. Findings from these studies have led to recommended protein intakes between 1.2 -1.6 g·kg BM · during energy balance (24), and intakes of 1.8 – 2.7 g·kg^{-1} BM ·have been proposed for the athletic population during ER (23). However, the vast majority of these studies have been examined in either active males or overweight females. The optimal intake required for LBM retention during ER weight loss in athletic females is not yet clear. Therefore, more research is warranted on dietary protein intake necessary to exhibit LBM preservation during ER in females.

1.3 Dietary weight loss interventions in the athletic population

To date, only one well-matched study has examined increased dietary protein intake on body composition changes in resistance trained males following a severe (40% reduction) short term high protein hypoenergetic diet on weight loss (17). In this study dietary protein intake of ~2.3g.kg.$^{-1}$ BM.d^{-1} (total energy intake %: 35% PRO, 50% CHO and 15% FAT) was shown to preserve more LBM compared with protein intake of ~1.0g.kg.$^{-1}$ BM.d^{-1}. Hence, these data support the notion that increased dietary protein consumption during a period ER spares the loss of LBM that is crucial for maintaining performance, particularly in athletes who are competing at the highest level. Furthermore, exercise performance was not compromised following ER in athletic males (17) which would be of interest to the athletic population where any diet-

induced changes in body composition and performance would be advantageous. Nonetheless, direct comparisons may not be directly applicable to active females, due to the difference in size and musculature between sexes. Females generally have lower BW and LBM comparable to male counterparts and it is not yet known the differences in protein requirements between sexes, therefore further research is warranted to identify optimal protein intake required for LBM preservation in active females during short term ER.

Therefore, the present study investigated the impact of high protein (HP) hypoenergetic diet compared with a normal protein hypoenergetic diet (CON) on body composition and exercise test performance in competitive active females.

We hypothesise that increased protein intake will reduce total body mass and fat mass, but ameliorate the loss of lean body mass when compared to normal protein intake. Furthermore, we hypothesise that maintenance of lean body mass will increase the power:mass ratio and improve exercise performance following increased dietary protein intake during a severe short term hypoenergetic diet.

Chapter 2: Methods

2.1 Participants

Active females between the ages of 18-35 years were recruited from local sports clubs and academies that represented, football, netball, rowing, muay thai and athletics. Twenty four participants were recruited, 18 completed the study. Four participants were not included due to inclusion criteria requirements. Two participants were removed during the diet intervention due to a lack of adherence to study guidelines. Participant inclusion criteria required a body fat percentage ≥15% body fat and habitual protein intake was to be ≤ 20%. Participant characteristics are displayed in Table 1. Participants were required to complete a minimum of three training sessions per week including sport-specific training. Participants received an information sheet detailing the purpose, possible risks and benefits of the six week study before written informed consent was obtained. The School of Sport Research Committee (SSREC) at the University of Stirling and the East of Scotland NHS Research Ethics Committee (NHSREC) approved this study.

Table 1: Participant characteristics and anthropometric measurements at baseline

	Control (n=9)	High Protein (n=9)	P Value
Age (Yrs)	21 ± 4	20 ± 2	0.45
Body Fat (%)	27.9 ± 2.4	26.8 ± 7.3	0.66
Body Mass (kg)	67.3 ± 9.57	66.6 ± 11.1	0.89
Height (cm)	172.3 ± 2.63	169.1 ± 2.3	0.38
Training (hrs) per week	10.3 ± 3.5	9.6 ± 2.0	0.67

Participant characteristics and anthropometric measurements at baseline. Values are expressed as Mean ± SD. No significant ($p \leq 0.05$) differences were found between groups at baseline.

2.2 Study Design

In a parallel group design, 18 active females were recruited to take part in the study. Participants were assigned to either a high protein group (HP) (n=9) or a control group (CON) (n=9). Participants were required to attend four separate testing sessions over

the course of the study. The overview of the study design is shown in Figure 1. The initial visit was used as a screening session to determine both a general health profile and baseline body composition values. This session was also used to familiarise the exercise protocols to ensure that all participants were aware and comfortable with all testing procedures. Participants returned for the second testing visit where baseline exercise performance tests were completed and participants were given a diet that equated to 100% habitual energy intake based on food diaries the participants completed. The third testing session was completed following the 7 day diet (100%) where body mass, body composition and exercise performance tests were completed (Pre). Following this, participants were given a 14 day diet which equated to 60% of habitual energy intake. Participants were divided into their allocated intervention groups. The first nine participants were randomly assigned to each group using block randomization, while the second half were allocated by training load and matching anthropometric results. Participants returned to the laboratory for the final testing session (Post). Body mass, body composition and exercise performance test results were measured to evaluate any changes following the two week diet intervention. Participants were encouraged to maintain their habitual training protocol throughout the duration of the six-week study.

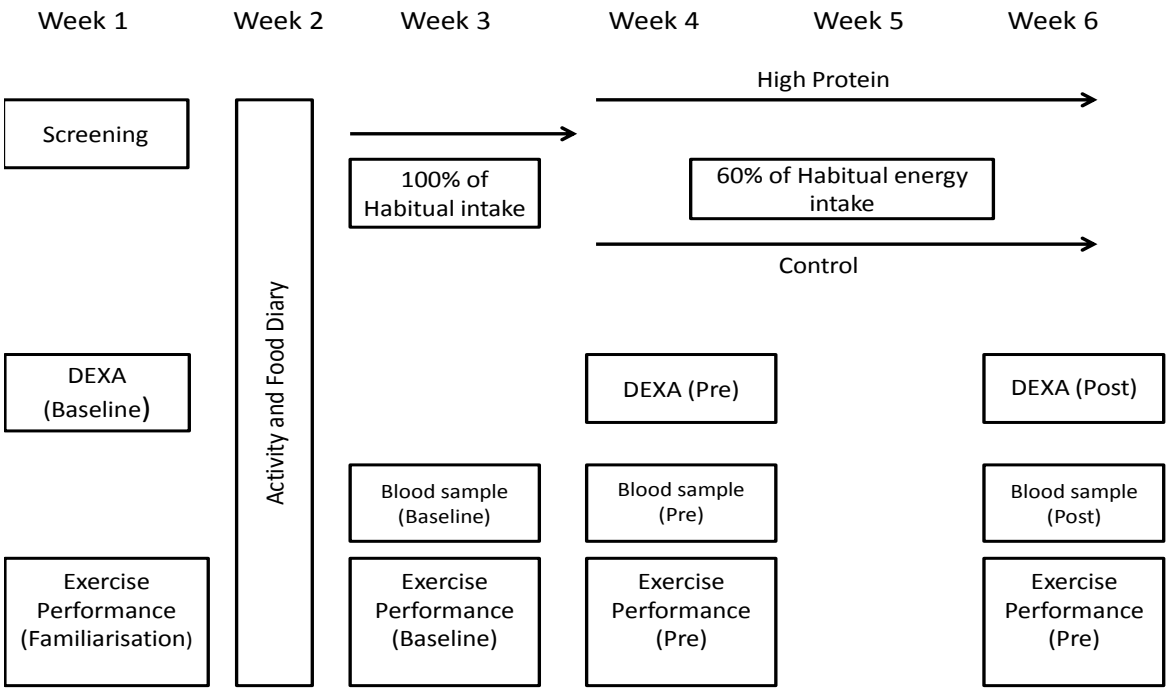

Figure 1: Schematic overview of study protocol.

2.3 Diet Intervention

Following the familiarisation session participants were asked to complete 3 day food and exercise dairies. All food and drink consumed was weighed and recorded over three separate days; a rest day, training day and a competition day which was analysed using dietary analysis software for habitual energy intake (Microdiet V2). Participants were also asked to log all exercise sessions completed in a training diary and asked to maintain a similar schedule throughout the duration of the study. After baseline testing participants were provided with a 7 day diet with a macronutrient composition to represent 100% of their habitual energy intake. All participants were instructed not to eat or drink anything else other than what was provided. The only exception was the *ad libitum* consumption of water. Following the second testing session (Pre) participants were given a 14 day diet which represented 60% of habitual energy intake.

Participants were randomly allocated to the intervention groups of high protein (HP) or control (CON). The macronutrient composition of the CON group constituted 15% protein, 35% fat and 50% carbohydrate. The HP diet constituted 35% protein, 15% fat and 50% carbohydrate. The energy and macronutrient intake of both groups is described in Table 2. Mean daily protein intake for the HP group during the 2 wk intervention was 112 ± 18 $g \cdot d^{-1}$, equivalent to 1.71 ± 0.31 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$. The CON group consumed 56 ± 9 $g \cdot d^{-1}$, equivalent to 0.86 ± 0.23 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$. Protein intake was evenly distributed throughout the day with 3 meals and snacks. The composition of all diets was customised to the individual's habitual intake. However, in an attempt to enhance compliance, diet was modified if the participants disliked particular foods that were prescribed. Group allocation was not disclosed to the participants. Participants received an additional food log diary and electronic scales during the 14 days to report any other drinks or foods that were consumed or any prescribed food that was not consumed. Participants were asked to report back immediately so that the macronutrient compositions could be modified accordingly the following day.

Table 2: Diet composition of food during prescribed diets.

	Week	Control (n=9)	High Protein (n=9)	P Value
Energy	Week 3	2114 ± 517	2173 ± 343	0.84
(K)	Weeks 4-6	1347 ± 264	1302 ± 209	0.64
CHO (g)	Week 3	283 ± 54 (4.32 ± 1.26)	272 ± 46 (4.15 ± 0.83)	0.65 (0.73)
($g \cdot kg^{-1}$ BM $\cdot d^{-1}$)	Weeks 4-6	176 ± 32 (2.68 ± 0.78)	168 ± 26 (2.58 ± 0.49)	0.58 (0.74)
FAT (g)	Week 3	83 ± 31 (1.27 ± 0.61)	79 ± 21 (1.20 ± 0.30)	0.76 (0.74)
($g \cdot kg^{-1}$ BM $\cdot d^{-1}$)	Weeks 4-6	51 ± 13 (0.77 ± 0.26)	24 ± 5 (0.38 ± 0.09)	**<0.01** (<**0.01**)
PRO (g)	Week 3	101 ± 25 (1.56 ± 0.55)	108 ± 25 (1.65 ± 0.41)	0.59 (0.69)
($g \cdot kg^{-1}$ BM $\cdot d^{-1}$)	Weeks 4-6	56 ± 9 (0.86 ± 0.23)	112 ± 18 (1.71 ± 0.31)	**<0.01** (<**0.01**)

Diet composition of food during prescribed diets. Values are expressed as mean ± SD. Values in bold show significant difference between groups ($p<0.05$). CHO= Carbohydrate, Pro= Protein. 3 Day food diaries were recorded prior to the Pre testing trial. Week 3 represents 100% prescribed habitual diet energy intakes. Weeks 4-6 represent 60% hypoenergetic diet energy intakes.

2.4 Testing Day Protocol

Participants were required to complete four separate testing visits over a 6-week period. On testing days participants were asked to report to the laboratory before 8.00am following an overnight fast. Participants were asked not to complete any vigorous exercise 24 hours prior to the testing day. This was to reduce potential changes in muscle glycogen levels following exercise which may alter body composition readings and exercise test performance scores. Participants were also asked to not eat anything after 22.00pm the evening prior to the testing day. Participants were asked to consume 500ml of water in the morning in order to control hydration levels (25). Upon arrival in the morning of a testing session, participants provided a urine sample. A blood sample was collected from the antecubital vein (visit 2,3 and 4) into three 5mL vacutainers containing ethylenediaminetetraacetic (EDTA), acidserum (untreated) and lithium heparin (LH). Participants had their stature and body mass measured in light clothes on a laboratory scale (SECA digital scales) to the nearest 0.1 kg. Subjects then completed a body composition scan.

2.4.1 Body Composition

A whole body Dual energy X-ray absorptiometry (DEXA) (Lunar, GE Healthcare Prodigy, GE Healthcare, Buckinghamshire, UK) scan was used to determine body composition at visit 1 (Baseline), 3 (Pre) , and 4 (Post). The DEXA measured various whole body composition values: total mass (TM), fat mass (FM), fat free mass (FFM) lean body mass (LBM) and body fat percentage (BF). Participants were asked to wear minimal clothing consisting of a sports bra and a pair of shorts and were asked to wear the same clothes at each subsequent testing day. A pregnancy test was completed before the scan to ensure no participants were pregnant due to the small levels of radiation from the scan. The scan patient position was standardized and maintained throughout the duration of the study. Following this, participants completed a battery of tests.

2.4.2 Exercise Performance

Participants underwent a five-minute standardised warm up before completing a 6 second Wingate test, a maximal isokinetic leg strength test, 20 m sprint and finally the Yo-Yo anaerobic endurance test. These tests were selected to cover four different components of exercise. All machine settings were set during visit 1 and remained constant throughout the study for each participant.

2.4.2 Wingate test

A 5 min warm up was completed on a cycle ergometer (Excalibur Sport; Lode, Netherlands). Participants included 5x5-s sprints during the final 2 min of the warm up before moving onto the testing ergometer. The 6-s Wingate test was performed on an electronic ergometrer (Excalibur Sport; Lode) using the official Wingate software (Wingate version 1.0.13; Lode, Netherlands) and recorded maximal anaerobic power output and peak power. Prior to the test participants completed a 1 min lead in to increase cadence from 70 to 100 revs/min. Participants were encouraged to pedal at maximal effort maintaining the highest possible cadence for the full 6-s. A 5 min cool down was then performed on the ergometer.

2.4.2 Isokinetic Strength

Participants completed an isokinetic peak force test on the electromechanical Kin Com Isokinetic Dynamometer (Kin-Com 125). Participants were seated in the testing chair with 90° of hip and knee flexion. Participants were secured into the seat through stabilization straps. The axis of the dynamometer was then aligned with the anatomical axis of the knee joint for each participant. Participant's dominant leg was used for testing and was assessed from 20° knee flexion to 70° knee flexion.
Five submaximal warm-up muscle contractions of 2–4-s were performed; two at 50%, two at 70% and one at 90% of participant's perceived maximal contraction. Each contraction was separated by 30-s of rest. The testing protocol was explained to each participant. Participants performed at least three maximal contractions of $60°\cdot s^{-1}$ and $120°\cdot s^{-1}$. Each maximal concentric contraction was followed by a maximal eccentric contraction separated by a 5-second pause. Following a 30-s interval the next maximal contraction was completed. The highest concentric and eccentric scores were

accepted as the maximal voluntary contraction. Participants were allowed a two minute rest interval before testing began at 120°·s^{-1}. Participants were vocally encouraged throughout the trials and were asked to grip the sides of the testing seat for comfort.

2.4.2 Speed and Yo-Yo Endurance Test

The 20m sprint and Yo-Yo endurance tests were completed on hard tennis courts. Participants were given 5 minutes to complete a dynamic warm up of their choice that was repeated for each trial in order to complete three maximal sprints. In the final 2 min of the dynamic warm up participants completed five sub-maximal 20 m sprints; two at 50%, two at 70% and a final sprint at 90% perceived maximum speed. Two minutes of rest were allocated before participants began maximal 20 m sprint testing. Each participant completed three maximal sprints separated by a one minute active recovery in between sprints. The best score out of the three sprints was used. Sprint time was recorded through electronic speed gates (Brower Timing) set at 0m, 5m and finally 20m. Participants were set 1m behind the timing gates and were encouraged to sprint maximally through the final gates at 20m. Following sufficient rest participants began the Yo-Yo level 1 recovery test. The test is instructed by a pre-recorded cd and consists of incremental shuttle runs. Cones were placed 20 metres apart, the participant ran 20 metres and turned at the cone and returned back to the starting point when signalled by the recorded beep from the cd. A period of 10-s was allocated for active recovery between every 40 metre (out and back) shuttle. When the subject failed to touch the starting line in time of the beep on two continuous shuttles the test was terminated. All testing conditions were kept the same and each subject completed each test individually to remove the element of competition.

2.5 Blood analysis

Blood samples were collected from antecubital vein into three 5 ml vacutainers containing ethylenediaminetetraacetic (EDTA), acidserum (untreated) and lithium heparin (LH). Blood samples were centrifuged at 4°, 3500rpm for 10 min within 2 hrs of collection. Plasma stores were transferred into 1 mL containers and stored at -80°until analysis. Samples were collected on three occasions, visit 2 (Base), 3 (Pre) and 4 (Post).

Blood progesterone concentrations were determined by a commercially available ELISA kit (Eagle Biosciences) through a 4 parametre logistic curve to determine the concentration which then highlighted the stage of the menstrual cycle the participant was in during the study.

2.6 Statistical analysis

Statistical analysis was performed on Minitab (Minitab17 statistical software). Two sample t-tests were used to detect any significant differences between macronutrient intakes in both intervention groups during the 100% and the 60% diet intervention weeks. Paired t tests were used to run the time effect within groups at Base-Pre. 2 sample t-tests were used to detect any changes between groups at Pre and were performed on all exercise test and body composition values. General linear model repeated analysis of variance (ANOVA) was conducted with comparisons between Pre to Post diet intervention on all exercise performance and body composition measures in order to test for a treatment and time interaction between the HP and CON groups. All results were represented as mean ± one standard deviation (SD) unless otherwise stated. Tests were considered significant with a P value of ≤ 0.05.

Mechanistic inferential statistics were completed to generate magnitude-based inferences about the population values and probabilities of effect in Pre to Post measurements with 90% confidence limits (CL). The benefit of using these statistics is that it emphasizes effect magnitudes and estimates precision rather than an absolute effect vs no effect as seen by the conventional null hypothesis significance testing based on $p<0.05$ (25). It also quantifies the probability of an important effect with suitable inferential descriptors to assist interpretation. Thresholds for inferences remained constant at 0.2. Using the Compare 2 means spreadsheet (8) the effect statistic was calculated. The effect statistic relates to the mean effect calculated as the difference between HP and CON for the change in value between Pre and Post intervention. The likelihood of the outcome being substantially beneficial or negative as well as trivial was determined using the same published spreadsheet (8). Likelihoods

were determined as: <0.5%; very unlikely, 0.5-5%; unlikely, 5-25%; possibly, 25-75%; likely, 75-95%; very likely, 95-99.5%; and most likely, >99.5%.

Chapter 3: Results

3.1 Body composition

3.1.1 Baseline to Pre Diet Intervention

No significant differences were observed in groups between Base-Pre or between groups (Pre) in any of the body composition measurements as displayed in Table 3. No significant changes were detected in body mass from Base-Pre in both groups (CON, p=0.57 HP, p=0.09), no significant change was detected at Pre intervention between groups (p=0.82). Fat mass was not different over time (Base-Pre) (CON p=0.97, HP p=0.58) or between groups at Pre intervention (p=0.79). No differences were observed in lean mass between the groups at Pre intervention (p=0.92), and furthermore, no differences were observed from Base-Pre in either group (CON, p=0.43, HP, p=0.56).

Table 3: Body composition values measured at baseline and immediately before the dietary intervention.

Variable	Intervention Group	Baseline	Pre Diet Intervention	Difference	P Value In group (Base-Pre)	P Value Between groups (PRE)
Body Mass (kg)	Control	67.2 ± 9.6	67.4 ± 9.4	0.2 ± 0.8	0.57	0.82
	High Protein	66.6 ± 11	66.3 ± 11.4	-0.3 ± 0.4	0.09	
Fat Mass (kg)	Control	18.1 ± 3.5	18.1 ± 3.3	-0.0 ± 0.8	0.97	0.79
	High Protein	17.5 ± 7.1	17.4 ± 7.4	-0.1 ± 0.8	0.58	
Lean Mass (kg)	Control	46.3 ± 6.3	46.5 ± 6.3	0.2 ± 0.7	0.43	0.92
	High Protein	46.4 ± 6.3	46.3 ± 6	-0.1 ± 0.6	0.56	

Body composition values measured at baseline and immediately before the dietary intervention. Values are means ± SD. (CON n=9, HP n=9). No significant interactions were found in groups (Base-Pre) or between both diet intervention groups at Pre.

3.1.2 Pre –Post Diet Intervention body composition measurements.

Figure 2 displays mean changes in total body mass, fat mass and lean mass (kg) from Pre to Post measurements. Reliability of the dexa calculated from intraclass correlation coefficient and was 0.99 for TBM, FM and LBM. No significant differences were observed between groups in body composition measurements following diet intervention. Further data analysis using magnitude-based inferential statistics revealed that increased dietary protein intake was unlikely (13%) harmful and possibly (52%) substantial positive in terms of attenuating (1.0 kg; CL ± 0.6) the loss of LBM after 2 weeks of a hypoenergetic diet. The decrease in LBM was greater in the CON group (-0.4 ± 1.1kg, (36% of TBM)) compared with the HP group (-0.10 ± 0.7kg, (10% of TBM). Differences in body segment composition (legs and trunk) were observed at Pre-Post diet intervention as summarized in Table 4.

Independent of dietary protein intake, reductions in total body mass and fat mass were observed in both intervention groups. Greater fat mass loss was reported in the HP group (-0.9 ± 0.4kg, (90% of TBM)) in comparison to the control group (-0.7kg ± 0.6, (64% of TBM)) however no significant differences were detected between the two treatment groups (p=0.43).

No significant differences were observed in total body mass loss between the groups (p=0.85), however the control group displayed greater losses following the two week diet intervention (CON, -1.1 ± 1.1kg vs HP,-1.0 ± 0.7kg), respectively. Individual lean mass and fat mass differences over the two week diet intervention (kg) are plotted in Figure 3a and 3b. In both groups 5/9 individuals lost LBM while the other four in each group reported gains in LBM. All bar one participant decreased FM in the CON group while all HP participants reported FM loss following the two week intervention.

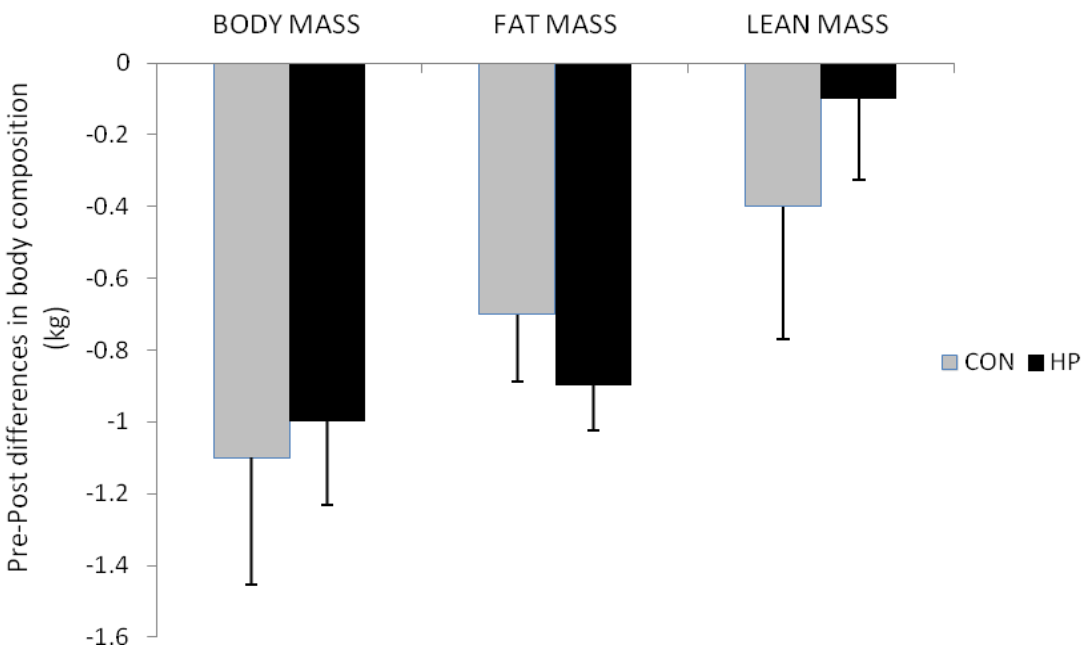

Figure 2: Pre to Post diet intervention changes in total body mass (TBM), fat mass (FM) and lean body mass (LBM) measurements (kg) assessed by dual-energy X-ray absorptiometry following a two week hypoenergetic (60% of habitual energy intake) diet. Values are represented as mean ± SEM. CON, control. HP, high protein. CON n=9, HP n=9. No significant treatment×time interactions were found between intervention groups.

Table 4: Pre-Post intervention differences in body composition measurements in both intervention groups.

	Diet Intervention		P Value	[c] Qualitative Inferences			
Anthropometric measurements	CON	HP		[a] Mean effect statistic [b] ± 90% CL (kg)	Substantially Positive (%)	Trivial (%)	Substantially Negative (%)
Pre-Post differences (kg)							
Body Mass (kg)	-1.1 ± 1.1	-1.0 ± 0.7	0.85	0.1; ± 0.64	36 possibly	41 possibly	23 unlikely
Fat Mass (kg)	-0.7 ± 0.6	-0.9 ± 0.4	0.43	-1.5; ± 0.7	10 unlikely	30 possibly	60 possibly
Lean Mass (kg)	-0.4 ± 1.1	-0.1 ± 0.7	0.55	1.0; ± 0.6	52 possibly	35 possibly	13 unlikely
Leg Fat Mass (kg)	-0.2 ± 0.3	-0.2 ± 0.1	0.85	-1.0 ± 0.60	22 unlikely	43 possibly	34 possibly
Leg Lean Mass (kg)	-0.1 ± 0.56	-0.2 ± 0.4	0.92	-0.0 ± 0.65	26 possibly	40 possibly	33 possibly
Trunk Fat Mass (kg)	-0.5 ± 0.3	-0.6 ± 0.4	0.39	-2.2; ± 0.87	83 likely	12 unlikely	5 very unlikely
Trunk Lean Mass (kg)	-0.1 ± 0.54	0.1 ± 0.60	0.72	1.0 ± 0.79	15 unlikely	28 possibly	57 possibly

Pre-Post diet intervention differences in body composition measurements in both intervention groups. Values are mean ± SD. (CON n=9, HP, n=9). CON, control. HP, high Protein. No significant treatment×time interactions were found between both diet intervention groups. [a] Mean effect statistic was determined by the difference between HP and CON for the change in value between Pre and Post testing. [b] ± 90% CL: add and subtract this number to the mean effect to obtain the 90% CI for the true difference. [c] Qualitative Inferences based on probability of effect being substantially beneficial or negative as well as trivial. Likelihoods were determined as: <0.5%; very unlikely, 0.5-5%; unlikely, 5-25%; possibly, 25-75%; likely, 75-95%; very likely, 95-99.5%; and most likely, >99.5%.

Figure 3a: Pre-Post diet intervention differences in lean body mass (LBM) expressed on an individual participant basis. (CON n=9, HP, n=9). CON, control. HP, high protein.

Figure 3b: Pre-Post diet intervention differences in fat mass (FM) expressed on an individual participant basis. (CON n=9, HP n=9). CON, control. HP. High protein.

3.2 Exercise Test Performance

3.2.1 Baseline to Pre diet intervention exercise performance test scores.

There was a statistically significant change in isokinetic concentric strength at both 60°·s^{-1} and 120° s^{-1} over time (Base-Pre) in the CON group (60° s^{-1}, p=0.01, 120° s^{-1}, p=0.01), however no significant changes were found between groups at Pre (60° s^{-1}, p=0.08, 120° s^{-1}, p=0.28). No significant changes over time were reported at Base-Pre in both groups or between groups at Pre for eccentric values at 60° s^{-1} and 120° s^{-1} as presented in Table 5.

Yo-Yo scores improved significantly in the HP group over time (Base-Pre) (p=0.03) no change was detected in the CON group (p=0.73), however no significant change was observed between groups at Pre intervention testing (p=0.20).

No significant differences were observed in groups from Base-Pre or between groups (Pre) in sprint performance (5m, p=0.57, 20m, p=0.46) or anaerobic power (p=0.93).

Table 5: Base and Pre diet intervention exercise performance scores.

Variable	Intervention Group	Baseline	Pre Diet Intervention	Difference	P value In group (Base-Pre)	P value Between groups (PRE)
5m Sprint (secs)	Control (n=7)	1.12 ± 0.02	1.09 ± 0.02	-0.03 ± 0.02	0.28	0.57
	High Protein (n=6)	1.11 ± 0.05	1.11 ± 0.03	0.00 ± 0.03	0.83	
20m Sprint (secs)	Control (n=7)	3.50 ± 0.04	3.46 ± 0.04	-0.04 ± 0.02	0.08	0.46
	High Protein (n=6)	3.54 ± 0.14	3.54 ± 0.11	0.00 ± 0.10	1.00	
Kin Kom Concentric 60°	Control (n=7)	356 ± 33	400. ± 27.	44 ± 13	**0.01**	0.08
	High Protein (n=6)	344 ± 34	335 ± 22	-9 ± 31	0.79	
Eccentric 60°	Control (n=7)	439 ± 55	483 ± 70	44 ± 51	0.41	0.29
	High Protein (n=6)	431 ± 56	400 ± 33	-31 ± 35	0.40	
Concentric 120°	Control (n=7)	290 ± 22	339 ± 33	49 ± 15	**0.01**	0.28
	High Protein (n=6)	283 ± 37	297 ± 23	14 ± 32	0.67	
Eccentric 120 °	Control (n=7)	411 ± 50	509 ± 62	98 ± 54	0.11	0.32
	High Protein (n=6)	461 ± 65	435 ± 38	-26 ± 39	0.51	
Wingate peak power (W)	Control (n=7)	987 ± 65	1042 ± 61	55 ± 48	0.30	0.93
	High Protein (n=6)	1029 ± 69	1034 ± 59	5 ± 29	0.86	
Yo-Yo Distance (m)	Control (n=7)	691 ± 58	714 ± 92	23 ± 63	0.73	0.20
	High Protein (n=6)	907 ± 150	996 ± 169	89 ± 34	**0.03**	

Base-Pre exercise performance test scores in both the HP and CON group. Values are expressed as mean ± SEM. CON, control. HP, high protein. Secs, seconds, N= Nanometres, W= Watts, M= Metres.

3.2.2 Exercise performance Pre to Post diet intervention test scores.

No statistical differences were observed between intervention groups in any of the exercise performance tests from Pre to Post diet intervention testing. Table 6 illustrates Pre-Post exercise performance scores.

3.3.2 Anaerobic Power (Wingate)

The CON group mean scores increased (25 W ± 48) whereas the HP group decreased peak power (-4 ± 32W) following the two week diet intervention, however no statistical changes were detected between groups (p=0.60).

3.3.2 Isokinetic Strength

Both intervention groups increased strength in all four isokinetic muscle contractions from Pre-Post. The HP group reported greater mean increases in maximal concentric contractions at 60° s^{-1} and 120° s^{-1}, however no statistical differences between groups were observed (60° s^{-1} p=0.09, 120° s^{-1} p=0.17) Conversely, the control group reported greater increases in maximal eccentric contractions at 60° s^{-1} and 120° s^{-1}. No treatment×time interactions were observed between groups (60° s^{-1} p=0.66, 120° s^{-1} p=0.52).

3.3.2 Sprint Performance

No statistical differences were detected between groups in the 5m sprint performance from Pre to Post testing (p=0.69). CON group participants reported faster scores in the 20 m sprint in comparison to the HP group however no treatment×time effect was detected (p=0.50).

3.3.2 Anaerobic endurance (Yo-Yo)

Increased protein intake had no effect on anaerobic endurance. Both intervention groups reported similar increases in distance covered (m) in the Yo-Yo test (CON, 75 ± 61m, HP, 71 ± 37m) from Pre to Post diet intervention testing but no significant difference between groups was observed (p=0.96).

Table 6: Pre and Post diet intervention exercise performance test scores.

Variable	Diet Intervention	Pre Diet Intervention	Post Diet Intervention	Difference	P Value (T×T)
5 m Sprint (s)	Control (n=7) High Protein (n=6)	1.09 ± 0.02 1.11 ± 0.03	1.09 ± 0.03 1.10 ± 0.04	0.00 ± 0.03 -0.01 ± 0.03	0.69
20 m Sprint (s)	Control (n=7) High Protein (n=6)	3.46 ± 0.04 3.54 ± 0.11	3.44 ± 0.07 3.56 ± 0.10	-0.02 ± 0.03 0.02 ± 0.04	0.50
Kin Com Concentric 60° (N)	Control (n=9) High Protein (n=9)	400 ± 27 335 ± 22	423 ± 29 411 ± 28	23 ± 23 76 ± 19	0.09
Eccentric 60° (N)	Control (n=9) High Protein (n=9)	483 ± 70 400 ± 33	558 ± 54 448 ± 49	75 ± 48 48 ± 33	0.66
Concentric 120° (N)	High Protein (n=9) Control (n=9)	339 ± 33 297 ± 23	332 ± 24 327 ± 22	7 ± 16 30 ± 20	0.17
Eccentric 120° (N)	High Protein (n=9) Control (n=9)	509 ± 62 435 ± 3.7	563 ± 54 451 ± 48	54 ± 54 16 ± 22	0.52
Wingate peak power (W)	Control (n=8) High Protein (n=9)	1042 ± 61 1034 ± 59	1067 ± 78 1030 ± 54	25 ± 48 -4 ± 32	0.60
Yo-Yo Distance (m)	Control (n=8) High Protein (n=9)	714 ± 92 996 ± 169	789 ± 123 1067 ± 169	75 ± 61 71 ± 37	0.96

Pre-Post exercise performance test scores in both the HP and CON group.
Values are expressed as mean ± SEM. CON, control. HP, high protein. Secs= seconds, N=Nanometres, W= Watts, M=Metres. No significant ($p \geq 0.05$) treatment×time interactions were found in any of the exercise performance tests from pre to post intervention diet testing.

3.3 Blood progesterone analysis

No statistical changes were observed between groups in progesterone concentration during the diet intervention (P=0.22) as shown in figure 4. Individual progesterone concentrations are plotted below in Figures 5a and 5b. In the HP group 3 participants were in the luteal phase (LP) of the menstrual cycle during the 2 week intervention with increased progesterone concentrations. The remaining participants remained in the follicular phase (FP) throughout. In the CON group all participants were in the FP during the 2 week intervention.

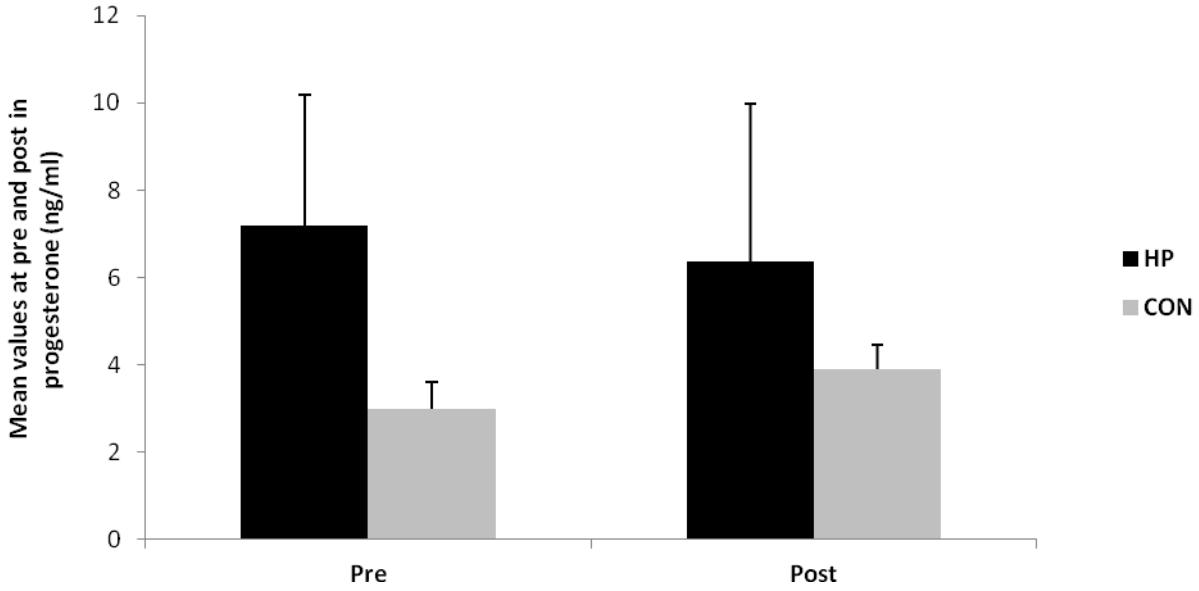

Figure 4: Mean values of progesterone concentration at pre and post in both intervention groups. Values are Mean ± SEM. CON, control, HP, high protein. CON n=5, HP n=7. No significant (p≥ 0.05) treatment×time interactions were from pre to post intervention diet testing.

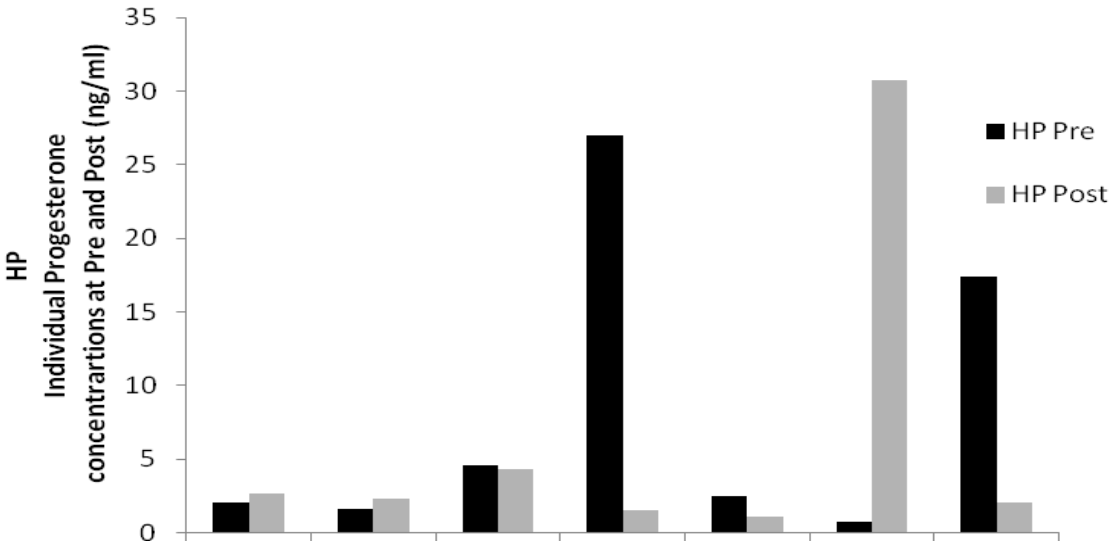

Figure 5a: Individual blood progesterone values (ng/ml) at both pre and post during the two week hypoenergetic (60%) diet intervention in the HP group. N=7.

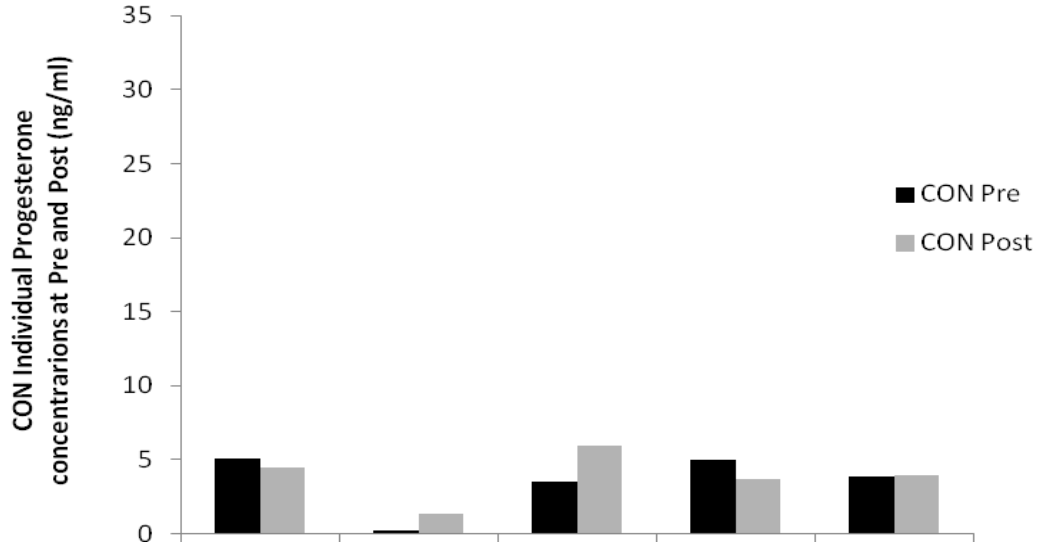

Figure 5b: Individual blood progesterone values (ng/ml) at both pre and post during the two week hypoenergetic (60%) diet intervention in the CON group. N=5.

Chapter 4: Discussion

The present study investigated the impact of increasing the dietary protein content of a hypoenergetic diet on whole body composition changes in total body mass, lean body mass and fat mass, alongside exercise performance in active females. In contrast to a similar study in trained young men (17), using a traditional null hypothesis statistical approach, the present study findings revealed no differences in any measurements of body composition or exercise performance parameters between HP and CON. Conversely, the more practical magnitude-based inferential statistics approach revealed a 52% 'possibly substantially positive' effect of increasing the protein content of a severe two week (40% reduction in habitual energy intake) hypoenergetic diet on the preservation of lean body mass. These data imply that the protocol may have the potential to increase the power: mass ratio if the period of hypoenergetic dieting had been prolonged, however warrants more research.

4.1 Body Composition

Previous studies in overweight and athletic populations have reported increased dietary protein intake ameliorates the loss of LBM during ER (14, 17). A similar study in athletic males reported a significant preservation of LBM following increased protein intake (2.3 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$) compared to lower protein intake (1.0 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$) following a severe (40% habitual energy intake) 2 week hypoenergetic diet (17). In contrast, in our hands, an increased dietary protein intake had no statistical effect on the preservation of LBM following 2 weeks of a hypoenergetic diet. These conflicting results between studies may be attributed to two main factors. First, a discrepancy in total daily protein consumption, expressed relative to body mass, was evident between studies. Whereas the prescribed macronutrient composition of the hypoenergetic diet was identical between studies (%total energy intake: 50% carbohydrate, 15% fat and 35% protein), the relative protein intake (2.3 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$) of trained males in the previous study (17) was considerably higher compared with the present study in active females (1.7 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$). In accordance, the consumption of increased dietary protein during ER has been associated with the greater preservation

of LBM, with intakes of 1.8-2.7 $g \cdot kg^{-1}$ BM $\cdot d^{-1}$ recommended for the athletic population (23). Hence, a plausible explanation for the failure to observe a significant effect of increased protein intake in active females may be attributed to the intake of dietary protein being insufficient to promote a significant preservation of LBM. A large degree of variability in habitual protein intake, when expressed as a percentage total energy intake, was observed in the present study. Indeed, three out of nine participants actually decreased their absolute (g) protein intake during the two week diet intervention. Therefore, we accept that it may have been more beneficial to examine intakes based on $g \cdot kg^{-1}$ BM$\cdot d^{-1}$ rather than percentage of overall macronutrient composition, given they are most accurately determined relative to body mass. Moreover, from a practical standpoint, the majority of studies present recommendations on a $g \cdot kg^{-1}$ BM$\cdot d^{-1}$ basis.

There is limited research on the impact of high protein intake on LBM retention in young active females. In contrast to the present study, the preservation of LBM loss was observed with intakes of 1.4 $g \cdot kg^{-1}$ BM$\cdot d^{-1}$ in recreationally active females during a four week weight loss diet (16). However, direct comparisons may not be applicable due to the training status of participants in the previous study (16). In the previous study (16), participants were excluded if they competed in sports and the habitual training hours of recruited females was considerably less than the participants in the current study. Thus, it may be the case that competitive athletic females in the current study require protein intakes above the previous recommendations of 1.2-1.6 $g \cdot kg^{-1}$ BM$\cdot d^{-1}$ (15). While the optimal protein requirement during weight loss in the female athletic population has not been determined, dietary protein intakes in excess of 2.0 $g \cdot kg^{-1}$ BM $\cdot d^{-1}$ have been shown to result in the preservation of LBM in males, during a severe acute period of weight loss (17). To expand the present study, a future study should be designed to investigate the impact of increasing dietary protein intake to ≥2.0 $g \cdot kg^{-1} \cdot d^{-1}$ based on recent recommendations (23) on body composition changes during short term and severe hypoenergetic dieting in active females.

A secondary factor that may explain the discrepant results between past and present studies is the differences in training modality. Mettler et al recruited male participants with at least 6 months of resistance training experience and whom currently

completed at least two resistance training sessions a week. Participants were asked to continue their habitual resistance training throughout the protocol (17). Conversely, performing resistance training was not a pre-requisite for eligibility in the present study and indeed only three out of eighteen participants in (HP n=2, CON n=1) performed resistance-based exercise training as part of their habitual training schedule. Participants maintained their regular training schedule throughout the duration of the study to limit any training adaptation interferences. The combination of resistance training coupled with increased protein intake may potentially explain the reasons why LBM loss was statistically different in higher protein groups comparable to normal protein groups in resistance trained males (17). It is well established that pairing resistance exercise with increased protein intake aids the preservation of LBM during negative energy balance (1). On a mechanistic level, the postprandial response of myofibrillar protein synthesis to protein ingestion is impaired during short-term energy restriction (20), however this response has been shown to be attenuated by combining resistance training with an increased protein intake (1). Since no clear sex-difference in the MPS response to exercise and protein feeding during energy balance has been observed in young adults it is plausible to assume that the basal MPS response to feeding alone would be similar between male and females with increased dietary protein during ER. We suggest that training did not provide any additional anabolic stimulus as seen in studies where resistance training was performed (17), furthermore had we included resistance training as part of the inclusion training status (6 month experience) we may have observed similar results of LBM retention or gains as seen in males following increased protein intake during ER (17). Future studies are warranted to investigate the impact of increasing protein intake in combination with resistance exercise on preserving LBM in active females.

Although the change in LBM after 2 weeks of a hypoenergetic diet was not statistically different between HP and CON using null hypothesis testing, the mean value loss of LBM was greater in CON (-0.4kg) compared with HP (-0.1kg). Of the ten participants that lost LBM during ER, the magnitude of LBM loss was ameliorated by increasing dietary protein intake. We employed magnitude-based inferential statistics to detect a potential effect and found that increased dietary protein intake was unlikely (13%)

harmful and possibly (52%) substantially positive in terms of attenuating (1.0 kg; CL ± 0.6) the loss of LBM after 2 weeks of a hypoenergetic diet. To our knowledge, the present study is the first to implement a magnitude-based inferential statistics approach alongside a conventional null hypothesis significance testing strategy. Mechanistic-based inference statistics were used to determine the mean effect statistic of increased protein intake on LBM loss (kg) through confidence based intervals to establish the likelihood of meaningful change. This analysis enabled us to estimate a potential beneficial effect rather than disregard the effect as the value was deemed not to be significant ($p \geq 0.05$). As such, this statistical outcome provides preliminary evidence, that increased protein intake (1.71 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$) may possibly attenuate the loss of LBM if applied to a longer term weight loss intervention in active females. Whilst, the current study only monitored body composition changes over a short period to enhance dietary compliance, it is plausible that, based on qualitative inferences and probability, if replicated over a longer time period we could potentially see significant LBM retention with higher protein intake comparable to lower protein. In support of this notion, recent literature reported greater LBM retention and gains consuming 1.4 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$ following longer term ER (10 weeks) in comparison to short term ER (5weeks) (6). Furthermore, attenuated LBM loss has been observed in studies following a longer term energy restriction spanning 10-16 wks (10, 13, 14). Given the potential benefit of increased protein on LBM preservation from the current analysis, these novel findings would be of key interest to the athletic population where the smallest changes in body composition could make a considerable difference in terms of improving exercise performance. Therefore, potential future studies should investigate the impact of increased protein (2x RDA) intake on LBM retention in active females over a longer period of time (*e.g.,* 10 weeks) using a similar study design.

Another key aspect to consider is differences in the composition of other macronutrients (carbohydrate and fat) between past and present studies. The preservation of LBM and greater loss of FM loss during an ER diet has been observed when protein intake was increased at the expense of carbohydrate (12, 13, 14). It has been suggested that a lower carbohydrate, high protein diet during ER leads to a greater lean to fat mass body composition (22). In the present study, carbohydrate

intake was maintained at 50% total energy intake and the protein intake was increased at the expense of fat. This dietary strategy was consistent with the previous study in trained young males (17). It is well established that reducing dietary carbohydrate intake during training leads to a decrease in muscle glycogen stores which in turn could alter training and competitive performance during ER (5). Hence, it was important to maintain carbohydrate intakes to allow full recovery of muscle glycogen stores and ensure that training/competition performance or dexa measurements were not compromised throughout the duration of the study. Further research should look at varied macronutrient compositions to identify weight loss strategies that both maintain LBM and exercise performance in athletic females during ER.

Several factors, including dietary protein source (type), timing and frequency have been known to affect LBM preservation during ER (23). Participants were prescribed foods similar to initial reporting in the food diaries to enhance compliance and adherence, consuming protein primarily from animal sources or dairy. However protein quality (type) was not controlled between groups. Recent data suggest that greater MPS stimulation is achieved following ingestion of animal protein comparable to other protein sources such as soy (3). Nonetheless, in this study there was no explicit focus on the quality of protein prescribed which may potentially explain why no statistical differences were detected in LBM maintenance between groups. In addition, it has been suggested that evenly spaced feedings of 20-25g replicated x4 throughout the day may potentially promote LBM preservation (2). Whilst participants protein consumption was distributed throughout the day, the timing of protein ingestion was not monitored and the frequency of feeding was not precisely scheduled in this study. Thus, potential future research could examine the importance of protein quality (type) and specific timing on body composition changes following increased protein during ER.

Most of the previous literature on high protein short term weight loss interventions in females has been conducted in postmenopausal women (10, 12, 13, 14) whilst there is little literature available in young active females whom are within their regular menstrual cycle. Fluctuations in the hormone progesterone are seen during the

menstrual cycle which is divided into two phases, follicular phase where progesterone is low ≤5 and the luteal phase where progesterone levels are elevated. It has been suggested that menstrual cycle stage could mildly affect body water particularly during the luteal phase (LP) where progesterone concentrations are elevated (26). This could potentially alter LBM body composition reading where water retention can occur during LP and increase LBM readings. However recent research found no changes in FFM during different phases of the same menstrual cycle (7). Although total body water was not measured in this study, participants' progesterone concentration was logged in the three week dietary period and over the 6 wk study each participant would have completed their regular cycle. Of the three participants that were reported in the LP stage in the HP group during the hypoenergetic diet weeks, only one participant increased LBM whilst the other participants that reported gains in LBM were in FP, suggesting that LBM was not explicitly altered by progesterone concentration.

4.2 Exercise Performance

The translation of diet induced changes in body composition to improved performance is of critical importance to the athlete. Consistent with previous findings in males (17), in the present study no substantial differences in any performance measures were observed between diet conditions. Indeed, none of the exercise performance parameters was substantially affected by protein intake following ER, suggesting that a 2 week hypoenergetic diet does not alter performance. Independent of protein intake, participants increased isokinetic strength following short term ER. Similarly both intervention groups reported greater anaerobic endurance. A potential explanation may be attributed to a better force to mass ratio due to TBM and FM reductions, which in turn would potentially improve lower body strength and endurance. Replicating the findings of recent work in wrestlers (18), we observed no significant changes in peak power output following increased protein intake. It also is plausible that participants potentially adapted to the testing protocol and in turn displayed a learning effect on exercise test parameters. Although participants were matched through training status, the group of participants were not homogenous in terms of

training status and sporting background. Indeed, participants were competing in different sports at different levels and as a result the performance tests were not sport specific. Therefore, further research should examine a more homogeneous group in a more sport specific manner to eliminate testing adaptation and variability.

4.3 Conclusion

In summary, the present study investigated the impact of high dietary protein compared to normal protein intake of a hypoenergetic diet on whole body composition changes in total body mass, fat mass and lean body mass, as well as exercise performance in active females. No significant differences were observed in body composition with high protein intake during energy restriction. Mechanistic-based inferential statistics suggest that 1.7 $g \cdot kg^{-1}$ $BM \cdot d^{-1}$ (35% PRO) dietary protein may be potentially beneficial and maintain LBM. Nonetheless, further research is warranted to identify more information on the dose relationship of increased protein intake on LBM loss and exercise performance during ER in active females.

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Appendices

Appendix 1 Pre-Participation Health Screen Questionnaire

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Pre-Participation Health Screen Questionnaire (PPHS-Q)

Date	Researcher	Signature

Accuracy in completion of the PPHS-Q is of the utmost importance

The purpose of the pre-participation health screen is:

To optimise safety during testing.

To identify medical risk factors which may prevent you from participating.

To identify those with special needs.

Name:_____Age:_____ Participant no:_____

Address_____
Gender:_____

_____Tel:_____(H)
_____(W)

Doctor's name:_____Tel:_____(W)

Doctor's address:_____
_____

Section A: Medical History Summary and Recommendations	
Date:	
Date:	
Date:	

Section B: Coronary Heart Disease Risk Index		Date	Date	Date
Group				
1. 1-10	No supervision required – exercise at will			
2. 10-17	No supervision required – use general exercise guidelines			
3. 18-27	No supervision required – use prescribed programme only			
4. 28-40	Use prescribed programme – Personal Training recommended			
5. 41+	Use prescribed programme – Personal Training and re-test within 8 weeks recommended			

SECTION A: MEDICAL HISTORY

Have you ever been told that you have had or have any of the following conditions?

If yes, please mark with an X in the appropriate box:

CARDIAC (Heart Related Diseases)

o	Heart Attack	o	High blood pressure
o	Coronary thrombosis (blood clot)	o	Rheumatic fever
o	Narrowing of arteries	o	Angina / Chest Pain
o	High cholesterol	o	Congenital Heart Disease
o	None		

Further / comments

PULMONARY (Lung Diseases)

o	Asthma	o	Exercise-induced asthma
o	Chronic Bronchitis	o	Emphysema
o	T.B.		
o	None		

Other / comments

OTHER

- o Type I Diabetes (insulin dependent)
- o Type II Diabetes (non-insulin dependent)
- o Anaemia (ion deficiency)
- o Rheumatic fever
- o Kidney disease
- o Angina / Chest Pain
- o Rheumatoid Arthritis
- o Congenital Heart Disease
- o None
- o Pregnant

Other / comments

ORTHOPAEDIC SURGERY (Musculo Skeletal)

Surgery

- o Neck
- o Hip
- o Back
- o Knee
- o Shoulder
- o Ankle
- o Arm
- o Foot
- o None

Other / comments

INJURY

Have you suffered any of the following injuries? If so, how long ago?

- o Neck vertebrae
- o Rotator cuff
- o Tennis elbow
- o ITB
- o Achillies Tendonitis
- o None
- o Back vertebrae
- o Impingement Syndrome (shoulder)
- o Runner's knee
- o Lower leg
- o Plantar Fascitis

Other / comments

MEDICATION

Do you use medication at present for any of the following? (If yes, please state the drug)

- o Heart rhythm
- o Blood clotting.
- o Asthma
- o Emphysema
- o Diabetes
- o Cholesterol
- o Kidney
- o Arthritis
- o Depression
- o None
- o Blood pressure
- o Blood circulation
- o Bronchitis
- o Flu
- o Thyoid dysfunction
- o Anaemia
- o Liver
- o Muscle injury

SECTION B: CARDIOVASCULAR DISEASE RISK INDEX

Please read the following questions carefully and answer each accurately. Mark your choice with an X.

History of heart attack or bypass surgery / angioplasty

0	o	None	5	o	1 – 2 years ago
2	o	Over 5 years ago	8	o	< 1 year ago
4	o	3 – 5 years ago			

Family history of heart disease

1 o No known history

2 o 1 relative with cardiovascular disease over the age of 60

3 o 2 relatives with cardiovascular disease over the age of 60

4 o 1 relative with cardiovascular disease under the age of 60

6 o 2 relatives with cardiovascular under the age of 60

8 o Heart – related sudden death:

- o Male, first degree relative before the age of 55
- o Female, first degree relative before the age of 65

Age / Gender Index			Smoking status		
0	o	Male / female under 30 years of age	0	o	None
1	o	30 – 40 years of age	1	o	Pipe
2	o	Female 40 - 50 years of age	2	o	1 – 10 cigarettes daily
3	o	Male 40 – 50 years of age	3	o	11 – 20 cigarettes daily
3	o	Female 50 – 60 years of age	4	o	21 – 30 cigarettes daily
4	o	Male 60 – 70 years of age	5	o	31 – 40 cigarettes daily
4	o	Male / female 60+ years of age	6	o	41 – 60 cigarettes daily
			8	o	+ 60 cigarettes daily
			State how long you have smoked for:		
			Years_____months_____		

How would you describe your bodyweight?			Total Cholesterol		
0	o	Ideal weight	0	o	< 5 mmol / L
2	o	0 – 5kg overweight	1	o	5.0 – 5.2 mmol / L
4	o	6 – 10kg overweight	3	o	5.3 – 5.9 mmol / L
6	o	11 – 15kg overweight	5	o	6.0 – 6.2 mmol / L
8	o	+ 15kg overweight	6	o	6.3 – 6.9 mmol / L
1 0	o	Underweight	7	o	7.0 – 7.5 mmol / L
			8	o	> 7.5 mmol / L
				o	Not sure

Systolic Blood Pressure		Diastolic Blood Pressure	
0 o	< 130 mmHg	0 o	< 80 mmHg
1 o	130 – 140 mmHg	1 o	81-90 mmHg
2 o	141 – 150 mmHg	2 o	91 – 100 mmHg
3 o	151- 160 mmHg	3 o	101 – 110 mmHg
4 o	> 160 mmHg	4 o	> 110 mmHg
o	Not sure	o	Not sure

Diabetes		Occupational activity level	
0 o	None	1 o	Intense physical labour
1 o	Type II (non-insulin dependent)	2 o	Moderate (walk often etc.)
2 o	Type I (insulin dependent)	3 o	Sedentary

Work Stress Tension

0 o No stress, very relaxed

1 o Moderate work stress and relaxed personality

2 o High work stress but cope well

3 o Very high work stress and tense personality

Physical Activity Status **(for a minimum of 30 minutes a session)**

1 o Exercise 4 or more times per week

2 o Exercise 2 – 3 times per week

3 o Recreational sport once a week

4 o Recreational sport occasionally or complete lack of exercise

SECTION C: LIFESTYLE

Do you participate in any of the activities more than twice weekly?

(Please tick all relevant activities)

- o Jogging more than 5 km
- o Cycling more than 45 min.
- o Swimming more than 600 m
- o Gym (Combined strength / aerobic)
- o Gym (weights only)
- o Gym (aerobic only)
- o Aerobic classes 45 min
- o Tennis 90 min
- o Squash 45 min.
- o Team sport (outdoor) – rugby hockey, soccer
- o Team sport (indoor) – basketball, netball, etc
- o Canoeing / Rowing 45 min

Do you have a regular menstrual cycle?

- o Yes
- o No

I have read, understood and completed this questionnaire to the best of my knowledge.

Signature: _____ Date: _____

Appendix 2: VOLUNTEER INFORMATION SHEET

UNIVERSITY of STIRLING

Scotland's University for Sporting Excellence

TITLE OF PROJECT: The impact of increased protein intake on body composition in active females during a hypoenergetic diet.

Researcher contact details:

Name: Miss Lee Alexander

Tel: 07805519543

Email: l.h.alexander@stir.ac.uk

Principal Investigator details:

Name: Dr Ian Walshe

Tel: 07745520939

Email: ian.walshe@stir.ac.uk

INFORMATION TO POTENTIAL PARTICIPANTS

What is the purpose of the project?

This study is being undertaken as part of postgraduate degree project, examining the effect of increased protein intake during two weeks of energy restricted diet.

Body weight is important for athlete performance in a range of sports. To achieve better performance, athletes usually restrict their energy intake to lose body weight and gain a better strength to body weight ratio. This improved strength to body weight ratio can improve speed, power and endurance performance. Typical dietary energy restriction/weight loss programs often lead to a reduction in body fat, but also a loss of muscle mass, which may affect strength and power. Increasing the protein intake, during energy restriction, may lead to a loss of body fat but also preserve muscle mass which could maintain strength and power.

Previous research has shown that a high protein, low energy (hypoenergetic) diet can maintain lean muscle during weight loss in male athletes. However, it is not known if the same results would apply in female athletes. Therefore, the aim of this study is to monitor the effect of a high protein, hypoenergetic diet in comparison to a normal protein hypoenergetic diet on body mass, body composition and performance in competitive female athletes.

Why have I been selected to take part?

You have been asked to participate because you are female and aged between 18 and 35 years and complete at least three training sessions a week. You do not currently ingest any protein supplements and your daily protein intake is below 20%.

What will I have to do?

The study will involve four visits to the University of Stirling laboratories over a six week period.

Visit 1 – Pre-screening and familiarisation session.
Visit 2 – Pre-diet assessments and control diet.
1 week control diet.
Visit 3 – Post control diet assessments and intervention diet.
2 week calorie restricted diet.
Visit 4 – Post-intervention diet assessments.

Visit 1 - Pre-screening and familiarisation session.

You will be required to report to a University of Stirling laboratory in the morning (approximately 7-8 am) where details of the study will be outlined to you. You can use this opportunity to ask any questions you have about the study. If you feel you still would like to take part, you will complete a consent form and a health questionnaire. Height and weight will also be recorded. You will then provide a urine sample for a pregnancy test before a body composition scan. The scan which is a quick, painless body scan identifies bone density and body composition such as muscle mass and body fat through duel energy x-ray absorptiometry (DEXA). During the scan, you will be exposed to a very low dose of radiation (the equivalent of less than one day exposure to natural background radiation in the UK). You should not take part in the study if you are pregnant or plan to become pregnant during the study, as additional radiation exposure is not recommended. If you find that you have become pregnant during the study, you should immediately tell the researcher.

Following the health screen and body composition scan, you will be introduced to a battery of exercise tests to assess power, strength, speed and aerobic endurance. We will measure

power using a peak power test, involving cycling as hard as you can for 6 seconds. We will measure maximum strength on an isokinetic dynamometer. Speed will be measured using 20 metre sprint test. Finally, a shuttle test (Yo-Yo level 1) will assess anaerobic endurance. During the Yo-Yo shuttle test, you will run 20 meter shuttles at progressively increasing speeds until you can no longer keep at the set speed. Following this, you will be given a food diary and be asked to record all food and drink over a period of three days during the remainder of the week.

Visit 2 – Pre diet assessments and control diet.

During the second visit, you will also be asked to provide a urine sample (in private). You will then be asked to perform the exercise tests, used in the previous visit (visit 1). Following this visit, you will be provided with all of your food to consume. This diet will equate to 100% of your normal energy intake (based on the food consumption recorded in week 1). We will ask you to weigh all food that was leftover on the food scales which will be provided. We will ask you to maintain your normal training routine throughout the period of the study.

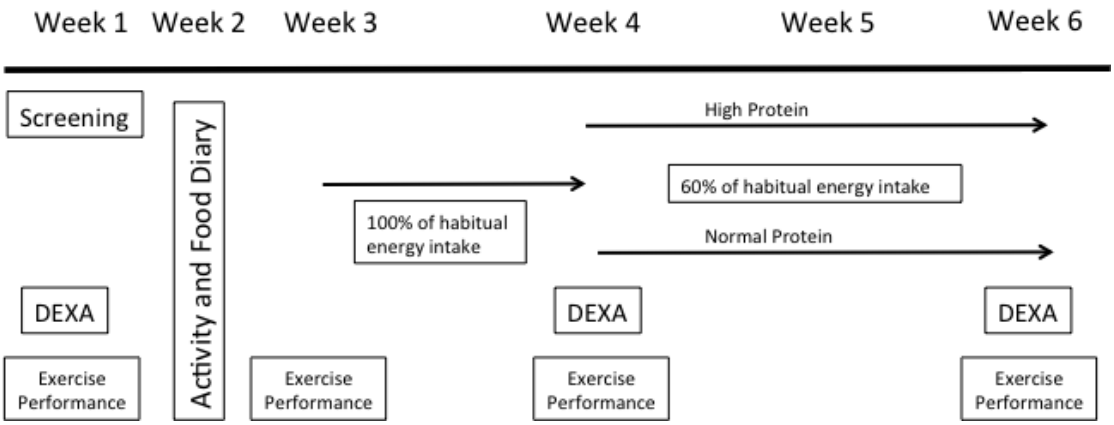
Visit 3 – Post control diet assessments and intervention diet.

The following visit (visit 3), at the end of the week, you will be asked to return to the laboratory to undergo a DEXA scan to assess any differences from the control diet. You will then perform the exercise test protocol to assess any performance measures from the control diet.

For the following two weeks, you will be given foods to consume which is part of a calorie restricted plan. This will equate to 60% of your normal energy intake. You will be allocated to either a high protein diet (35%) or a normal protein diet (15%) group. You will be asked not to eat anything else other than the food that is provided and to report the amount of food consumed at each meal.

Visit 4 – Post intervention diet assessments.

At the end of the two week period, you will be required to visit the laboratory (visit 4) for a final DEXA scan to assess for any differences in body composition. You will also undergo the exercise test protocol to assess for any differences in performance. An overview of the study protocol is outlined below.



Pre-visit preparation.

During the 24 h period before each of the trial visits, we will ask you not to do any heavy or prolonged exercise as this may affect exercise test scores. You will also be asked not to eat from 10 pm the night before each visit. However, you may drink water or take any necessary medication during this period.

What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

You should not take part if you have any kind of metabolic disorder. You should also not take part if you have very low body fat or do not have a regular menstrual cycle. If you are pregnant or seeking to become pregnant, you should not take part in the study. Exclusion criteria also include individuals who are currently or have recently been involved in drug or alcohol abuse.

Are there any risks?

There will be some minor risks from taking part in the study; however, if you are in good health, there should be no harmful effects from the two week calorie restricted diet. You will be exposed to radiation during the DEXA scans; however, this is a very small dose (the equivalent of less than one day exposure to natural background radiation in the UK for each scan).

Will my participation involve any physical discomfort?

During the exercise tests, you may feel some discomfort associated with high intensity exercise. You may also feel some sensations of tiredness and hunger from the calorie restricted diet which may impair your physical performance.

Will my participation involve any psychological discomfort or embarrassment?

No psychological discomfort or embarrassment will be caused.

Will I have to provide any bodily samples (i.e. blood, saliva)?

You will be asked for a urine sample on every visit prior to the body composition scan for a pregnancy test. You will also have a blood sample taken on visits 2, 3 and 4.

How will confidentiality be assured?

You will only be known by a number to protect anonymity. All data will be kept in a locked cabinet or computerised and accessed via a password which will be done in accordance with the Data Protection Act.

Who will have access to the information that I provide?

Only the investigating team will have access to information that you provide. All records will be kept confidential except for review by representatives of the University of Stirling Ethics Committee and NHS Research Ethics Committee. If your DEXA scans reveal any abnormal results (eg. low bone density), we will contact your General Practitioner to follow up these findings with the appropriate tests. We will inform you before contacting your General Practitioner.

How will my information be stored / used in the future?

All data will be kept in a locked cabinet or computerised and accessed via a password which will be done in accordance with the Data Protection Act. Data may be used for publication in the form of a scientific paper, presented at a conference or both. Any data used cannot be linked to you.

Has this investigation received appropriate ethical clearance?

Yes, the study and its protocol have received full ethical approval from the University of Stirling School Ethics Committee and NHS Research Ethics Committee.

Who has reviewed this study?

The East of Scotland Research Ethics Service REC 2, which has responsibility for scrutinising all proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from the University of Stirling Ethics Committee and NHS Tayside, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

Will I receive any financial rewards / travel expenses for taking part?

No financial reimbursement will be given for taking part. However, if you wish, we can send you a report of your body composition, exercise test data and dietary intake.

Can I withdraw from the project?

If you wish to withdraw from the project, you can inform the researcher or principal investigator by email, telephone or in person. You can withdraw from the project at any point without providing reasons for doing so and without prejudgment. Following withdrawal, all of your data will be removed from the data set. However, it may no longer be possible to withdraw your data once it has been fully anonymised. In this instance, data cannot be linked to you. If you do have any concerns about the study, please let the researcher or the principal investigator know.

If you wish to register a complaint about the study, please contact:

Dr Angus Hunter
Director of Research

University of Stirling
Tel: 01786 466497
Email: a.m.hunter1@stir.ac.uk

If I require further information who should I contact and how?

If you would like further information on the study please contact the researcher or principal investigator on the contact telephone or email listed above.

Appendix 3: Informed Consent Form

Informed Consent Form

UNIVERSITY OF STIRLING

TITLE OF PROJECT: Effects of a high protein hypoenergetic diet on weight loss, body composition and exercise test performance in competitive female athletes.

Participant number: _____

I have read and understood the Participant Information Sheet.

I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.

I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.

I understand that my General Practitioner may be informed if any unusual observations are made.

I agree to take part in this study.

I would like to receive feedback on my own performance at the email

Email address: _____

Signature of participant: _____

Printed name: _____ Date:

Signature of researcher: _____

Printed name: ___________________________________ Date: __________________

Appendix 4: Recruitment Poster

HEALTHY FEMALE COMPETETIVE ATHLETES REQUIRED!

- **Are you aged between 18-35 years old?**
- **Do you compete in a sport and train 3 times a week?**

We are looking for volunteers to participate in our new study, monitoring the effects of a high protein (35%) low energy diet on body composition changes in exercise performance.

- **Free gold standard DEXA scan**
- **Free food supply for three weeks**

Be a part of exciting new research!

Contact: Lee Alexander Email:l.h.alexander@stir.ac.uk