Exploring protein and macronutrient intakes in lean bodybuilders during caloric restriction

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed	
Date	

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LIST OF ABBREVIATIONS

CI Confidence intervals

CL Confidence limits

DALDA Daily Analysis of Life Demands of Athletes

FFM Fat free mass

HPLF High-protein low-fat

IGF-1 Insulin-like growth factor 1

IMTP Isometric mid-thigh pull

MPMF Moderate-protein moderate-fat

NBAL Nitrogen balance

POMS Profile of Mood States

SD Standard deviation

TMDS Total mood disturbance score

CHAPTER 1 – INTRODUCTION

Nearly a decade ago in 2004 I became interested in and eventually began competing in drug-free bodybuilding and strength sport. As my love of these sports grew, I found I had interest not only as a competitor but also as a coach and trainer. Since then I have been developing my career, both in the field and academically. Today, I have achieved professional status as a drug-free bodybuilder, write articles for lay and academic strength and bodybuilding publications and I have had the honour of working with hundreds of competitors from rank beginners to world champions. The science behind the training and nutrition for bodybuilding and strength is as much of a passion of mine as its practice.

In my studies I have found that sometimes professional and academic publications support the traditional training and nutrition practices in bodybuilding and strength circles, but often there are disparities. Sometimes I find that the available research is lacking in scope and that the recommendations by the authors are based on incomplete or inappropriate data. However, other times I find that some traditional practices persist despite a lack of evidence to support them.

As I gain more success in my field I am able to reach a wider audience with my articles and publications. In these articles I often highlight the instances where traditional practice should be updated to better inform athletes and trainers alike. Now in my post graduate education, I have the unique opportunity to study the areas where the research is lacking in scope and where there is contention between what is published and what is practised.

One of the greatest points of contention among the bodybuilding culture and scientific literature is protein intake. It is common to see recommendations of 1 to 2 grams per pound (2.2-4.4g/kg) in American bodybuilding magazines; however, the recommended range in most sports

nutrition literature for strength and hypertrophy is half that, falling in the range of 1.2-2.2g per kilogram (Wilson & Wilson, 2006). Bodybuilders may give more credence to the recommendations made in magazines rather than the scientific literature, as evidenced by some reporting they consume as much as 4.3g/kg of protein on average (Kim, Lee, & Choue, 2011).

I believe a large part of this disparity arises from the fact that bodybuilders undergo extended caloric restriction in order to compete and make the most dramatic changes in their appearance, achieving extremely low body fat levels. Bodybuilders believe that during periods where calories are restricted and body fat levels are low, higher protein intakes are critical for maintenance of muscle mass. Seeing the greatest changes in their physiques coinciding with high protein intakes likely influences their day-to-day dietary habits and thus the nutritional practice of the entire bodybuilding community.

While not all practices and beliefs of bodybuilders are supported by published empirical data, there is evidence that the contributions of dietary and body proteins to energy expenditure are increased when body fat percentage is low (Elia, Stubbs, & Henry, 1999; Forbes, 2000; Hall, 2007a) and that high-protein diets may be more effective for preventing muscle loss while dieting compared to traditional protein recommendations (Mettler, Mitchell, & Tipton, 2010; Walberg et al., 1988). However, no published guidelines for protein intake have yet to differentiate protein recommendations relative to body composition. Furthermore, while some review authors acknowledge that protein requirements change based on energy intake (Phillips, Moore, & Tang, 2007; Phillips & Van Loon, 2011; Tipton & Wolfe, 2004), robust recommendations for protein intake under these conditions are lacking. Questions remain as to the various potential effects of protein modification during caloric restriction. We do not definitively know how much protein should or should not be increased during caloric restriction nor how much and to what degree carbohydrate or fat should be lowered to accommodate an increase in protein.

Purpose statement

The purpose of this thesis is to assist with bridging the gap between what bodybuilders have practised based on observation and what nutritional science has sparsely investigated. With the paucity of research on calorically-restricted lean athletes and high-protein diets, we do not have a complete picture of what, how much, and when to recommend certain nutritional approaches in a definitive way. Thus, the goal of this thesis is to fill in some of these gaps in knowledge, to add context to the discussion and finally to provide practical nutritional guidance for resistance-trained athletes and bodybuilders alike during caloric restriction.

Study aims

The aims of this research were as follows:

- To review the published literature on protein intake during caloric restriction in athletic, non-overweight populations and its impact on changes in performance and anthropometrics.
- 2. To investigate the effects of two different protein intakes during caloric restriction on strength, anthropometrics and mental state in resistance-trained participants; one protein intake representative of common practice among bodybuilders and strength athletes, and the other representative of current sports nutrition guidelines.
- To synthesise the data collected in the review and the intervention study with the existing body of knowledge to provide nutritional guidance to strength athletes during caloric restriction and bodybuilders during contest preparation.

Study limitations

- The recommendations in Chapter 2 (systematic review) should be considered the current, most logical interpretations in an evolving field.
 The paucity of research available that met the inclusion criteria (six studies) prevents the conclusions from being definitive.
- 2. Participants in the intervention study were expected to follow the meal plans provided and to consume the nutrient powder provided daily.

Participants were also asked to continue their habitual training which was not supervised by the investigators. While participants were encouraged to truthfully report when deviations occurred and a non-judgemental relationship was emphasised, full compliance with the intended diet and their habitual training could not be guaranteed.

- 3. The reported changes in fat free mass (FFM) in Chapter 3 (intervention study) are based on prediction equations. While measurements of the changes in body mass, skin folds and girths themselves are reliable, the resulting values when they are used in prediction equations carry an added element of error.
- 4. Chapter 2 prescribes protein recommendations based on FFM due to the recommendation of a peer reviewer. However, protein intake is prescribed based on total body mass in Chapter 3 because this recommendation was made after data collection was completed.
- 5. While a double-blind design was applied to the intervention study, complete blinding of participants could not be guaranteed. In addition to meal plans, participants were provided with carbohydrate and protein or protein-only powders labelled "A" or "B". Armed with the knowledge that one intervention was moderate protein, and the other high protein, conceivably a participant could have looked up the nutrition information of their meal plans, infer which powder they were consuming and thus which intervention they were on.
- The length of the intervention study was two weeks for each caloricallyrestricted period. Often fat loss reduction periods last longer in fitness and sporting practice.
- 7. The caloric restriction (60% of habitual caloric intake) was high in comparison to recommended sports nutrition practice, thus the results may not be representative of what would occur with lesser energy restriction or a longer restriction period.
- 8. The final chapter presents much needed recommendations to an athletic population that is sorely lacking evidence-based guidelines. In large part, this lack of guidance exists because there is very little research in drug-

free bodybuilders or comparable populations. While the interventional study in this thesis included resistance-trained athletes it did not specifically include bodybuilders. Therefore, the guidance should be seen as an important step in promoting evidence-based practice but also preliminary in nature.

Study delimitations

- 1. To the best ability of the researchers the intervention study was doubleblind and thus bias from the participants or researchers was minimised.
- For the intervention study a 40% caloric restriction was chosen to allow for better comparison to the only other studies in this area of investigation, all of which incorporated the same level of restriction (Mettler et al., 2010; Pasiakos et al., 2013; Walberg et al., 1988).
- The statistical approach used in the intervention study is based on inferential statistics rather than null hypothesis testing. Instead of using statistical significance and P values, this approach uses magnitude-based inferences based on probabilities. Inferences are determined by where the confidence interval (CI) lies relative to thresholds for effects rather than the null value, and are referred to as mechanistic or clinical effects. Clear clinical effects are reported as being beneficial, harmful, or trivial, depending on the value of the effect. If the CI overlaps substantial levels of benefit or harm, the effect is described as being unclear. Inferences are also given qualitative probabilities that reflect the uncertainty of the value (e.g., possibly beneficial; very likely harmful). These chances are converted to descriptors as follows: <1%, almost certainly not; 1-5%, very unlikely; 5-25% unlikely or probably not; 25-75% possible or may be; 75-95%, likely or probably, 95-99%, very likely; >99%, almost certainly. For all variables that were measured, thresholds used to determine the magnitude of effect sizes were based on a modified Cohen's scale. Standardised thresholds of <0.2, <0.6, <1.2, and <2.0 were interpreted as trivial, small, moderate, and large effects, respectively. See the methods section of Chapter 3 for a more specific

explanation of the statistics used for that specific analysis. Readers unfamiliar with this statistical paradigm and its merits are referred to Batterham and Hopkins (2006) and Hopkins et al., (2009) for a more indepth discussion.

Thesis format and paper contribution

My thesis is presented as a series of chapters, which after this chapter includes a mixture of original research and reviews at different stages of publication. The second chapter is a systematic review of protein intake in lean calorically-restricted resistance-trained athletes which is currently under review with the International Journal of Sports Nutrition and Exercise Metabolism. This review is the first of its kind in that I examined protein intake specifically in non-overweight, resistance-trained populations during caloric restriction. Furthermore, I devised recommendations relative to FFM allowing a more individualised protein intake, which is rare in the published literature.

The third chapter comprises a double-blind crossover study on non-overweight, resistance-trained males. In this chapter I examined the effects of two isocaloric diets on strength, anthropometrics and mental state. One diet achieved a high protein intake via a reduction in fat, and the other provided a moderate intake in both protein and fat. This chapter is presented in the format of a manuscript submission to the Journal of Science and Medicine in Sport.

The fourth and final chapter comprises an overall discussion of the topic, tying together the data from the previous chapters with the existing body of knowledge to provide nutrition guidelines for bodybuilders during contest preparation. An excerpt from this chapter appears in a review article that has been submitted to the Journal of the International Society of Sports Nutrition. This chapter concludes the thesis as a whole. It is intended to present practical recommendations based on the overall body of evidence, i.e., both from the existing literature as well as the outcomes from my

intervention study. The contributions to the papers by the authors are as follows:

Paper 1:

A systematic review of dietary protein during caloric restriction in resistancetrained lean athletes: a case for higher intakes.

Helms, E., (80%), Zinn, C., (10%), Rowlands, D., (5%) & Brown, S., (5%)

Paper 2:

Physiological effects of protein modification in resistance-trained males during caloric restriction: a double-blind crossover study.

Helms, E., (80%), Zinn, C., (5%), Rowlands, D., (5%), Naidoo, R., (5%) & Cronin, J., (5%)

Paper 3:

An excerpt from, Evidence-based recommendations for natural bodybuilding contest preparation: Nutrition and supplementation.

Helms, E., (80%), Aragon, A., (10%) & Fitschen, P., (10%)

Finally, it should be noted that because this thesis is presented as a series of chapters, of which each is or is a part of a standalone publication, a certain amount of repetition is unavoidable. Two key repetitious areas include the introduction of Chapters 2 and 3 (which are similar because the same rationale drives both publications) and the recommendations for protein intake in Chapters 2 and 4 (as bodybuilders during contest preparation are calorically-restricted, resistance-trained lean athletes).

CHAPTER 2 - A SYSTEMATIC REVIEW OF DIETARY PROTEIN DURING CALORIC RESTRICTION IN RESISTANCE-TRAINED LEAN ATHLETES: A CASE FOR HIGHER INTAKES.

Summary

Caloric restriction occurs when athletes attempt to reduce body fat or make weight. There is evidence that protein needs increase when athletes restrict calories or have low body fat. Purpose: The aims of this review were to evaluate the effects of dietary protein on body composition in resistancetrained athletes during caloric restriction and to provide protein recommendations for these athletes. Methods: Database searches were performed from earliest record to July 2013 using the terms protein, and intake, or diet, and weight, or train, or restrict, or energy, or strength, and athlete. Studies included (N = 6) used adult (≥ 18 yrs) resistance-trained (> 6 months) humans of lower body fat (males ≤ 23% and females ≤ 35%) performing resistance training during energy restriction. Protein intake, FFM and body fat had to be reported. Results: Body fat percentage decreased (0.5% to 6.6%) in all study groups (N = 13) and FFM decreased (0.3 to 2.7kg) in nine of 13. Four groups gained or did not lose FFM. They had the highest body fat, smallest magnitudes of energy restriction or underwent novel resistance training stimuli. Two groups lost non-significant (p < 0.05) amounts of FFM. The same conditions that existed in the groups that did not lose FFM existed in the first group. These conditions were not present in the second group, but this group consumed the highest protein intake in this review (2.5-2.6g/kg). Conclusions: Protein needs for energy-restricted resistance-trained athletes are likely 2.3-3.1g/kg of FFM scaled upwards with severity of caloric restriction and leanness.

Key Words: body composition, strength training, metabolism, nutrition, strength, sport

Introduction

Caloric restriction during weight training is common among lean athletes attempting to make weight or improve body composition for competition. This situation frequently occurs among wrestlers, bodybuilders, power lifters, Olympic weight lifters, boxers and martial artists (Buford, Rossi, Smith, O'Brien, & Pickering, 2006; Mourier et al., 1997; Slater & Phillips, 2011; Umeda et al., 2004; Walberg et al., 1988). Despite the high frequency of observations of energy restriction by already lean athletes, studies in which these conditions are examined are rare (Garthe, Raastad, Refsnes, Koivisto, & Sundgot-Borgen, 2011; Mettler et al., 2010; Walberg et al., 1988). Protein guidelines to optimise body composition and performance during these periods have not yet been established.

Sport and nutrition scientists have supplied a range of recommendations for protein intake over the years. Differentiations in recommendations exist between endurance and strength athletes due to the metabolic demands of the sport and the adaptations desired from training (Butterfield, 1987; Lemon, 2000; Phillips, 2006; Phillips et al., 2007; Phillips & Van Loon, 2011). Less commonly, researchers point out that these requirements increase while athletes consume energy-restricted diets (Butterfield, 1987; Garthe, Raastad, Refsnes, et al., 2011; Mero et al., 2010; Mettler et al., 2010; Millward, 2004; Phillips & Van Loon, 2011; Stiegler & Cunliffe, 2006; Walberg et al., 1988).

When in negative energy balance, the efficiency of protein utilisation is enhanced (Saudek & Felig, 1976), which can be erroneously interpreted to mean that less protein is needed during weight loss. A more accurate explanation might be that this increase in efficiency is an adaptive mechanism to preserve FFM during starvation. When supply is limited, efficiency is increased, indicating the body's increased need for protein in states of negative energy balance (Fielding & Parkington, 2002). When significant weight loss occurs, FFM tends to be lost in greater amounts that correlate with the severity of energy restriction (Chaston, Dixon, & O'Brien, 2007; Garthe, Raastad, Refsnes, et al., 2011).

Slight energy deficits increase protein requirements which are further increased with exercise. Butterfield (1987) found that male athletes running 5-10 miles per day during a slight caloric deficit were in a significant negative nitrogen balance (NBAL), despite consuming 2g/kg of protein daily. Celejowa, et al., (1970) found that five out of 10 intermediate competitive weight lifters achieved a negative NBAL over the course of a training camp while consuming an average protein intake of 2g/kg, three of which were in a slight caloric deficit.

In addition to the presence of a caloric deficit, its magnitude has an impact on FFM changes as well. Greater caloric restriction (1100kcal/day versus 550kcal/day) can lead to declines in anabolic hormones and decrements in performance (Mero et al., 2010), and a smaller proportion of total mass lost coming from body fat (Garthe, Raastad, Refsnes, et al., 2011). Fast rates of weight loss in athletes with low body often result in FFM losses (Mettler et al., 2010; Mourier et al., 1997) and in some cases, coincide with decreases in performance (Buford et al., 2006; Umeda et al., 2004; Walberg et al., 1988).

Besides the presence and magnitude of an energy deficit, the availability of stored body fat also impacts changes in FFM (Elia et al., 1999; Forbes, 2000; Hall, 2007a). "Forbes' theory" states that during caloric restriction, reductions in body fat will increase the risk of FFM loss (Forbes, 2000; Hall, 2007a). There are significant differences in protein metabolism across subjects ranging from 6% to 50% body fat during negative energy balance. Elia et al., (1999) observed that subjects on the lower end of this spectrum derive two to threefold more energy from protein and excrete twice as much urinary nitrogen than subjects on the higher end. In the initial days of starvation, leucine oxidation increases among the leanest individuals but not among subjects highest in body fat.

FFM is more metabolically active than fat, and muscle gain is dependent upon skeletal muscle metabolism. Muscle is the site at which dietary protein aids resistance training adaptation; thus, optimal protein intake is likely relative to FFM. Therefore, protein intakes established by total

body weight result in higher protein intakes relative to FFM in obese subjects. This may help to explain why energy-restricted diets with comparable protein intakes (when established by total body weight) are more apt to produce FFM gains in overweight subjects performing resistance training (Demling & DeSanti, 2000; Stiegler & Cunliffe, 2006) compared to those at normal body fat levels (Mero et al., 2010). It also could be one reason as to why leaner subjects are more likely to lose FFM during energy-restricted diets (Forbes, 2000; Hall, 2007a; Stiegler & Cunliffe, 2006).

There are also significantly different endocrine responses between normal weight and obese individuals in response to energy deficits. Nair et al., (1987) found that unlike the morbidly obese (Suryanarayana, Kent, Meister, & Parlow, 1969), subjects of healthy weight experience a lowering of total testosterone production (-608.5 \pm 254.8nmol/L p < 0.05) and free testosterone (-30.5 \pm 11.1nmol/L p = 0.055) in response to fasting. A decline in this anabolic hormone could contribute to losses in FFM. Collectively the endocrine, metabolic, and body composition differences in lean versus overweight populations may indicate that lean dieters might benefit from a higher protein intake in an attempt to offset losses in FFM.

Traditional protein requirement studies have inherent methodological limitations. The most common technique used is NBAL; the process of comparing the amount of nitrogen entering the body via dietary protein, to that leaving the body via urine, faeces, sweat and other processes. Protein recommendations are based on the minimal intake required to prevent nitrogen losses. NBAL does not measure protein synthesis nor tissue specific breakdown (Nair et al., 1987; Oddoye & Margen, 1979). In two studies subjects were observed to maintain NBAL while losing FFM (Pikosky et al., 2008; Walberg et al., 1988). These discrepancies likely occur because NBAL tends to overestimate nitrogen intake, underestimate excretion (Kopple, 1987) and is inaccurate at high protein intakes showing impossible levels of retention (Lemon, Tarnopolsky, MacDougall, & Atkinson, 1992; Oddoye & Margen, 1979; Phillips, 2006; Tarnopolsky et al., 1992; Tarnopolsky, MacDougall, & Atkinson, 1988; Tipton, 2008).

The more modern technique of isotopic amino acid tracing can be used to track tissue-specific protein synthesis and breakdown (Zak, Martin, & Blough, 1979). However, most studies only measure synthesis (Wolfe, 2006). Phenylalanine tracing is often used as it is not synthesised endogenously or oxidised by muscle (Liu & Barrett, 2002; Smith, Villareal, & Mittendorfer, 2007), but is not without limitations (Marchini et al., 1993; Pikosky et al., 2008; Short, Meek, Moller, Ekberg, & Nair, 1999). Isolated amino acids may not represent the broad picture of protein metabolism, therefore multiple amino acids should be traced (Pikosky et al., 2008; Smith et al., 2007; Wagenmakers, 1999; Wolfe, Wolfe, Nadel, & Shaw, 1984). Even properly designed, tracer studies are acute in nature. They provide "snapshots" of protein turnover (Pikosky et al., 2008) and their results are not always indicative of long-term changes in FFM (Aragon & Schoenfeld, 2013; Pasiakos et al., 2013). This may be because amino acids have other impacts related to metabolic pathways and immune function (Phillips et al., 2007) and prior to oxidation exert a regulatory influence on maintenance and growth (Millward & Rivers, 1988, 1989).

Tracer data can be used to make mechanistic inferences but this methodology does not measure FFM or performance over time. NBAL can determine a minimum protein requirement, but what optimises accrual of FFM may be higher (Lemon, 2000; Phillips et al., 2007; Tipton & Wolfe, 2004; Wilson & Wilson, 2006). Establishing minimums is important, but sports nutrition should focus on determining intakes that optimise performance. Finding the optimal protein intake range during caloric restriction is especially valuable because if one macronutrient is set too high it can force another too low, potentially resulting in decreased performance (Mettler et al., 2010; Millward, 2004; Phillips et al., 2007; Tipton, 2011; Walberg et al., 1988). Therefore, to determine optimal intakes during caloric restriction, this review examines research that measures changes in body composition and performance over time.

To establish protein recommendations for resistance-trained athletes during weight loss, a review of the current body of knowledge on protein

intakes in energy-restricted athletes must be performed. This review examines the effect of protein intake on FFM when the subjects in question are: 1) engaged in regular weight training and have resistance training experience, 2) in a negative energy balance and 3) of a healthy or leaner body fat percentage (males 23% or lower and females 35% or lower) as defined by Gallagher, et al., (2000).

Methods

Search parameters and inclusion criteria

PubMed, MEDLINE, SPORTDiscus and CINAHL electronic databases were searched online. Various combinations of the keywords protein AND intake OR diet AND weight OR train* OR restrict* OR energy OR strength AND athlet* were searched in conjunction with limiting database results to academic journals, reviews and human subjects when applicable. Inclusion criteria were articles involving: (i) resistance-trained (six months experience or more); (ii) adults (at least 18 years old); (iii) of healthy or leaner body fat percentage (males 23% or lower and females 35% or lower); (iv) during caloric restriction; and (v) providing body fat percentages; (vi) FFM; and, (vii) dietary protein intake.

Exclusion criteria were articles that: (i) were only available as case studies, conference proceedings or in abstract from; (ii) did not involve participants performing regular progressive resistance training; (iii) included any ergogenic dietary supplementation; or, (iv) did not add to the progressive knowledge of the review by not consisting of original work or where the data were not reported. A comprehensive search through references and citation tracking on Google Scholar was used to identify any additional material. Following the search, two authors from the current review independently screened each article for inclusion. The screening process consisted of: (i) screening for duplicates; (ii) screening the title; (iii) screening the abstract; and, (iv) screening the full paper using the inclusion and exclusion criteria. If a discrepancy occurred between authors on the inclusion of a study, a third

author independently reviewed the article using the inclusion and exclusion criteria and a discussion occurred until a consensus was reached.

Assessment of study quality

Two authors from the current review independently assessed the methodological quality of each article. This assessment consisted of a 10-item custom methodological quality assessment scale (Table 1) involving a 20-point scoring system (ranging from 0-20) where 0 = clearly no; 1 = maybe, inadequate information or partially yes; and, 2 = clearly yes. Determining appropriate anthropometric measurements in item six follows the work of Ayvaz and Çimen (2011) where 0 = not appropriate or unknown; 1 = appropriate but performed incorrectly or with limitations; and, 2 = appropriate and correctly performed. This scale was designed to assess the methodological quality of studies examining anthropometric changes and was adopted from the qualitative scoring system utilised by Brughelli, Cronin, Levin and Chaouachi (2008). If consensus was not reached on an article's score by the two authors, a third author from the current review assessed the article in question to rectify differences and to help determine the final score.

Data extraction and analysis

Data were first extracted and categorised as body fat percentage and FFM in kilograms and then separated into groups by low-protein, high-protein, low-loss or slow-loss, high-loss or fast-loss and energy-restricted. Due to the heterogeneity of the study design and subject characteristics, data were not pooled together but instead analysed individually in a qualitatively descriptive method.

If standard deviations (SD) were not reported, data were imputed in as follows: (1) available SDs were individually squared; (2) summed and averaged; and, (3) square rooted to impute missing SDs. Similarly, missing p values were imputed as follows: (1) SD change of the mean was imputed based on similar study characteristics; (2) SD change of the mean was divided by the square root of the n to obtain the standard error of the mean change; (3) mean change was divided by the standard error of the mean change to

obtain the t-statistic; and, (4) a two-tailed Student's t-distribution was used to impute missing p values. Finally, mean differences (the mean of the post variable minus the mean of the pre variable) and 90% CIs were computed using the two-tailed inverse of the Student's t-distribution. All data were analysed using Excel (2010, Microsoft, Redmond, WA, USA) software.

Results

A large number of studies were located examining resistance training during weight loss with quantified protein intakes; however, the vast majority were performed with overweight participants. Among the studies located, nine were identified in which athletic and non-overweight participants performed resistance training during negative energy balance. The full texts were further analysed to determine if they fit the inclusion and exclusion criteria. Three studies did not fit the criteria and were excluded (Celejowa & Homa, 1970; Mourier et al., 1997; Pasiakos et al., 2013). One was excluded because body composition was not reported (Celejowa & Homa, 1970). A second was excluded because branch chain amino acid supplementation was used by one experimental group and not the others (Mourier et al., 1997). The final study was excluded because the participants were not required to have resistance training experience (Pasiakos et al., 2013). Figure 1 represents the search and selection process in a graphical flow chart.

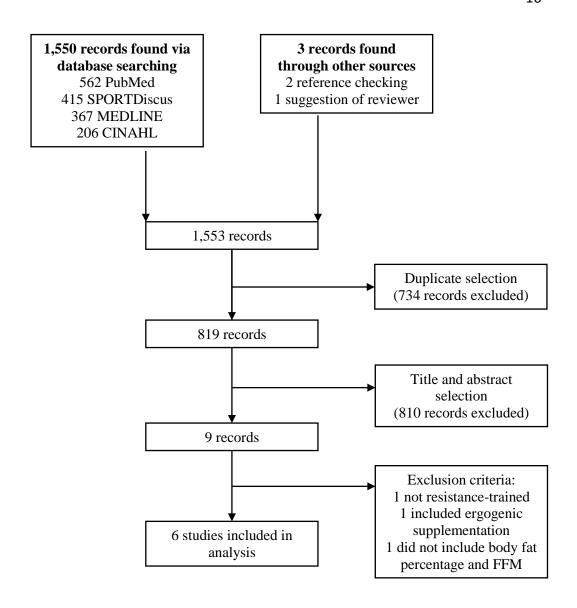


Figure 1. Search and selection process

In our methodological quality assessment of the six included studies there was a range of scores from 13 to 18 out of 20 with a mean score of 16. Of particular note was that in only one study was a power analysis for sample size calculation performed and in only half the studies was test-retest reliability performed on at least one of the included measurements. Scoring details of the studies are provided in Table 1.

Table 1. Methodological quality assessment

Quest	tion Criteria	Maestu et al., 2010	Mettler et al., 2010	Garthe et al., 2011	Walberg et al., 1988	Mero et al., 2010	Umeda et al., 2004
1	Power analysis was performed and justification of study sample size given.	2	0	0	0	0	0
2	Participant demographics were clearly defined: Gender, age, body composition and mass at the time of the test.	2	2	2	2	2	1
3	Participant characteristics were clearly defined: sport or activity and experience level at the time of the study.	2	2	2	2	1	2
4	Inclusion and exclusion criteria were clearly stated for participants.	2	2	2	1	2	1
5	Participants or groups of participants were similar at baseline or differences were accounted for and explained.	2	2	2	2	2	2
6	Anthropometric measurement methods were appropriate and discussion or conclusions acknowledged measurement limitations when applicable.	2	2	2	2	2	2
7	Methods were described in great detail to allow replication of the study.	2	2	2	2	1	1
8	Test retest reliability of measurement device(s) reported.	0	1	1	0	1	0
9	Outcome variables were clearly defined.	2	2	2	2	2	2
10	Statistical analyses were appropriate	2	2	2	2	2	2
	Total score out of 20	18	17	17	15	15	13

0 = clearly no; 1 = maybe, inadequate information or partially yes; 2 = clearly yes.

Participant populations included male and female adults with a mean age of 23.4 years. Training experience ranged from elite athletes and competitive bodybuilders to healthy adults performing resistance training. Table 2 outlines the subject and design characteristics of the studies included.

Table 2. Characteristics of studies of energy restriction in athletes undertaking resistance training

Study	Grou subje	ps and ects	Age (y)	Body mass pre study (kg)	Initial body fat (%)	Body fat assessment method	Energy deficit	Diet time	Protein intake g/kg/d	Prior training history	Training protocol
Walberg et al., 1988	CG LP HP	5 M 7 M 7 M	19.4 ± 0.7 21.4 ± 0.7 21.0 ± 0.9	74.6 ± 4.1 81.8 ± 2.9 80.2 ± 4.1	7.5 ± 1.2 11.4 ± 2.3 14.4 ± 2.3	UWW	n/a 51% baseline 51% baseline	1 wk	1.1 0.8 1.6	≥ 2 years weight training	Weights 6/d/wk
Umeda et al., 2004	CG LL HL	5 M 11 M 11 M	19.3 ± 0.6	78.7 ± 8.8 80.7 ± 13.1 78.5 ± 13.6	9.5 ± 8.2 11.4 ± 5.8 11.2 ± 6.6	UWW	n/a -2.4 kg BW -3.2 kg BW by study end	20 d	1.2, 1.4, 1.3 1.3, 1.0, 0.8 1.5, 1.1, 0.8 Day 20, 4, 1	College level judoists	Running and judo 6/d/wk and weights 2/d/wk
Maestu et al., 2010	CG ER	7 M 7 M	22.4 ± 3.4 28.3 ± 10.3	85.3 ± 10.5 82.2 ± 9.3	12.0 ± 3.4 9.6 ± 2.3	DXA	n/a 199 ± 115 kcal/d 536 ± 298 kcal/d 978 ± 625 kcal/d at start, middle and end	11 wk	1.7-1.9 2.5-2.7	Amateur level body builders	Habitual weights and aerobic training
Mero et al., 2010	SL FL	7 F 8 F	28.9 ± 6.2 28.0 ± 6.4	65.7 ± 4.0 66.9 ± 4.3	34.2 ± 4.0 31.8 ± 7.0	DXA	550 kcal/d 1100 kcal/d	4 wk	1.5 1.4	≥ 6 months weight and aerobic training	Habitual weights and aerobic training
Mettler et al., 2010	LP HP	10 M 10 M	25.8 ± 1.7 24.7 ± 1.6	78.3 ± 4.3 79.9 ± 2.9	17.4 ± 1.5 16.1 ± 1.6	DXA	60% baseline 60% baseline	2 wk	1.0 ± 0.0 2.3 ± 0.1	≥ 6 months weight training	Habitual weights and aerobic training
Garthe et al., 2011	FL FL SL SL	6 F 5 M 7 F 6 M	20.7 ± 6.4 20.9 ± 4.5 22.4 ± 3.1 24.9 ± 3.5	68.9 ± 6.7 81.9 ± 11.5 66.4 ± 8.8 78.5 ± 14.1	30.0 ± 5.0 16.0 ± 3.0 27.0 ± 5.0 17.0 ± 5.0	DXA	791 ± 113 kcal/d 469 ± 61 kcal/d	4-12 wk	1.4 ± 0.2 1.6 ± 0.4	Elite athletes from various sports	Normal sport training and weights 4/d/wk

Values are means ± SD when applicable or available.

M, male; F, female; CG, control group; ER, energy-restricted group; HP, high-protein group; LP, low-protein group; HL, high-weight loss group; FL, fast-weight loss group; LL, low-weight loss group; UWW, underwater weighing; DXA, dual-energy X-ray absorptiometry.

In all study populations body fat percentage was decreased by 0.5% to 6.6%. From Figure 2 and Table 3 it can be observed that only the female and slow-loss groups in Garthe et al., (2011) were able to both reduce body fat and increase their FFM (increases ranging from 0.6 to 1.1kg). In nine out of 13 study populations the FFM of participants was decreased with reductions ranging from 0.3 to 2.7kg (Figure 2). The fast-loss group in Mero et al., (2010) did not undergo a change in FFM and the slow-loss group underwent a non-significant decrease of 0.3kg. The participants in Maestu et al., (2010) experienced a FFM reduction of 0.4kg but this change was also non-significant.

Table 3. Changes to body composition achieved by energy restriction in athletes undertaking resistance training

trairiiig								
Study	Group		Body Fat (%)		Fat Free Mass (kg)			
		Pre	Post	Change	Pre	Post	Change	
Walberg	CG M	7.5 ± 1.2	6.6 ± 1.3	-0.9	68.9 ± 3.9	68.9 ± 3.7	0.0	
et al.,	LP M	11.4 ± 2.3	9.3 ± 1.7	-2.1	72.7 ± 3.1	70.0 ± 2.7	-2.7	
1988	HP M	14.4 ± 2.3	12.4 ± 2.3	-2.0	68.4 ± 4.0	67.0 ± 3.8	-1.4	
Umeda	CG M	9.5 ± 8.2	9.7 ± 4.5	0.2	70.9 ± 5.4	71.2 ± 5.6	0.3	
et al.,	LL M	11.4 ± 5.8	10.9 ± 6.4	-0.5	70.9 ± 8.6	69.2 ± 8.6	-1.7*	
2004	HL M	11.2 ± 6.6	10.5 ± 6.4	-0.7* [#]	68.9 ± 7.0	66.7 ± 6.4	-2.2* [#]	
Maestu	CG M	12.0 ± 3.4	11.8 ± 3.0	-0.2	70.5 ± 8.6	72.2 ± 7.8	1.7	
et al.,	ER M	9.6 ± 2.3	6.5 ± 1.5	-3.1*#	72.9 ± 8.4	72.5 ± 8.1	-0.4	
2010								
Mero et	SL F	34.2 ± 4.0	32.3 ± 4.6	-1.9*	40.6 ± 3.4	40.3 ± 3.8	-0.3	
al., 2010	FL F	31.8 ± 7.0	27.6 ± 7.9	-4.2* [#]	42.8 ± 5.4	42.8 ± 5.4	0.0	
Mettler	LP M	17.4 ± 1.5	16.4 ± n/a	-1.0	64.7 ± n/a	63.1 ± n/a	-1.6	
et al.,	HP M	16.1 ± 1.6	14.9 ± n/a	-1.2	67.0 ± n/a	66.7 ± n/a	-0.3#	
2010								
Garthe	FL F	30.0 ± 5.0	28.0 ± 4.3	-2.0*	44.6 ± 3.6	45.2 ± 3.6	0.6*	
et al.,	FL M	16.0 ± 3.0	13.3 ± 7.7	-2.7*#	65.5 ± 3.3	64.1 ± 6.8	-1.4	
2011	SL F	27.0 ± 5.0	20.4 ± 4.5	-6.6	46.3 ± 5.5	47.4 ± 5.1	1.1#	
	SL M	17.0 ± 5.0	11.9 ± 3.3	-5.1	62.3 ± 10.3	63.3 ± 10.3	1.0	

Pre and post values are means ± SD when available.

M, male; F, female; CG, control group; ER, energy-restricted group; HP, high-protein group; LP, low-protein group; HL, high-weight loss group; FL, fast-weight loss group; LL, low-weight loss group; SL, slow-weight loss group.

^{*} Significantly different from baseline value.

[#]Significantly different from comparative group(s).

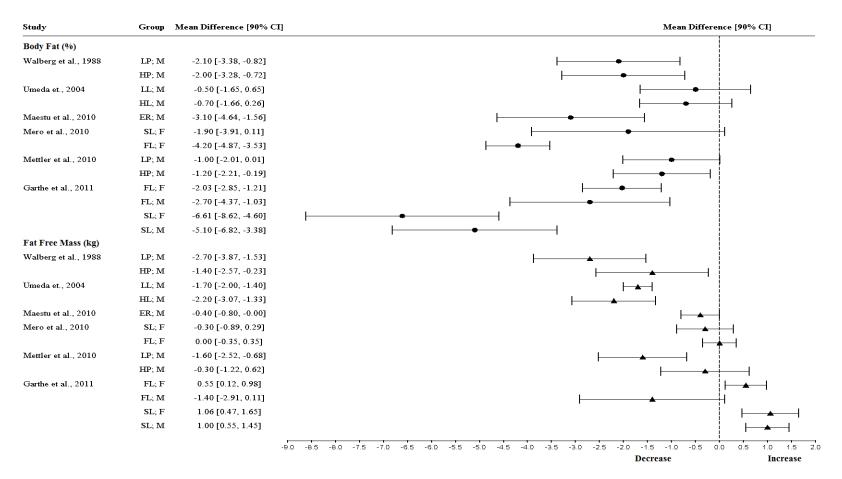


Figure 2. Forest plot summarising anthropometric changes.

Within each group presented as pre versus post (mean difference [90% CI]). M, male; F, female; ER, energy-restricted group; HP, high-protein group; LP, low-protein group; HL, high-weight loss group; FL, fast-weight loss group; LL, low-weight loss group; SL, slow-weight loss group; CI, confidence interval.

Discussion

The aim of this review was to establish protein recommendations for resistance-trained, lean participants who are restricting calories. Six published studies met the inclusion criteria for analysis while relevant manuscripts provided additional information and context. In addition to protein intake, the rate of weight loss, resistance training experience, and initial body fat levels may have a significant influence on changes in FFM and body fat when restricting calories.

The female athletes in Garthe et al., (2011) and female participants in Mero et al., (2010) who were able to avoid losses of FFM (actually gaining FFM in the former) had specific similarities which likely allowed this to occur. In Mero, et al., (2010) the women had the highest body fat percentage out of all populations included in this analysis and the female athletes in Garthe et al., (2011) had the second highest. Additionally, the slow-loss groups in both studies had the least aggressive energy restriction of all populations included. In contrast, the leanest men in Garthe et al., (2011) which were in the faster weight loss group experienced a loss of FFM. Another similarity between the two was the participants in Mero et al., (2010) were the least experienced resistance-trained population in this review and although the participants in Garthe et al., (2011) had prior weight lifting experience it was not a main component of their regular training. This may have contributed to the results, as novice weight lifters experience accelerated gains in FFM (M. D. Peterson, Rhea, & Alvar, 2005). Supporting this hypothesis, Garthe et al., (2011) noted that gains in FFM and performance came predominantly in the upper body and that the athletes already had a high volume of lower body training in their sport specific conditioning. Thus, the upper body may have experienced this novice effect. A follow-up study examining the long-term results in the same group of athletes six to 12 months later found the athletes had returned to their normal resistance training volume (half of that in the previous study) and their FFM had decreased back to baseline (Garthe, Raastad, & Sundgot-Borgen, 2011). Thus, it may be unrealistic to expect a lack of FFM loss or FFM

gain in leaner more experienced weight lifters at protein levels similar to Garthe et al., (2011) and Mero et al., (2010).

Like the publications by Mero et al., (2010) and Garthe et al., (2011), Umeda et al., (2004) examined high and low losses of body mass, but between groups of male judoists. However, unlike these studies, the participants were much leaner. As would be expected, the male judoists were a great deal leaner compared to the female participants of both Garthe (2011) and Mero's works (2010) and 5% to 6% body fat leaner than the male subjects in the study by Garthe (2011). This study examined judo competitors cutting weight for a competition; protein levels decreased over the length of the study as calories were decreased which can be seen in Table 1. The second and third highest amounts of FFM occurred in the high- and low-loss groups respectively. The high-loss group lost significantly more FFM than the low-loss group. However, making firm connections between the losses of FFM and protein intakes among the participants is difficult considering there was only a slight difference in protein intake between groups and more importantly due to the disparity in weight loss and thus energy intake between groups. What is clear, and what confirms the results reported by Mero (2010) and Garthe (2011), is that the magnitude of the caloric deficit imposed is likely one of the most powerful variables that impacts FFM loss, potentially being more important than protein intake.

In Walberg et al., (1988), the effects of two energy-restricted isocaloric diets of differing protein intakes were compared. Carbohydrate was reduced in the 1.6g/kg group to keep the interventions isocaloric. NBAL was negative in the 1g/kg group while it was positive in the 1.6g/kg group (despite losses of FFM). It should also be noted that the 1.6g/kg group displayed decreased muscular endurance compared to the 0.8g/kg group. The authors suggested this was possibly due to the caloric balance between the diets being established by a reduction in carbohydrate in the higher protein diet. This likely reduced muscle glycogen levels precipitating a reduction in muscular endurance. The authors noted this would likely compromise the effectiveness of the participants' habitual bodybuilding training characterised by high

volume and moderate repetition ranges. In regards to the anthropometric changes, the authors stated that with such a short time period of intervention (1 week), and considering the inherent 2% margin of error in hydrostatic weighing, conclusive changes to FFM were difficult to detect.

In a study by Mettler et al., (2010) the same basic premise and methodology was employed as in that of Walberg et al., (1988). However, different protein intakes were used, the subjects were not as lean and a larger number of measurements were taken. Unlike Walberg et al., (1988), the calorie balance between the diets was maintained by a reduction in dietary fat as opposed to carbohydrate. Performance and most blood parameters did not vary between the two groups. Unlike Walberg et al., (1988), the avoidance of carbohydrate restriction appeared to prevent reductions in performance. Similarly, the participants in Garthe et al., (2011) established the majority of their caloric deficit via a reduction in fat and all groups improved their one repetition maximum on squats, bench press, bench pull and their counter movement jump height. However, despite maintenance of performance in the high-protein group in Mettler et al., (2010), this group reported slightly but significantly reduced feelings of well-being as assessed by the Daily Analysis of Life Demands of Athletes (DALDA) questionnaire. It is unknown if this was caused by the increased intake of protein, the dietary fat reduction to allow for this increase or other factors. Therefore, while maintaining carbohydrate levels may aid performance, it is not known to what degree fat can be safely and pragmatically reduced. A comprehensive discussion of dietary fat in the context of dieting athletes is beyond the scope of this review. However, 20% of total calories which is the low end of some fat intake recommendations for resistance-trained athletes (Bird, 2010), may serve as a reasonable lower limit until more research is performed.

Maestu et al., (2010) observed non-significant losses of FFM in a group of drug-free bodybuilders consuming 2.5-2.6g/kg of protein during the 11 weeks prior to competition. When compared alongside the works by Walberg et al., (1988) and Mettler et al., (2010) and considering the 11-week time frame, it may seem that the higher the protein intake, the lower the chance

for FFM loss. However, it should be noted that this study did not include a low protein control. Furthermore, two subjects did lose significant amounts of FFM (1.5kg and 1.8kg), and the authors noted that these specific bodybuilders were among the leanest of the subjects. These two subjects lost the majority of their FFM (approximately 1kg) during the latter half of the intervention as their percentage of calories from protein increased from 28% to 32% and finally to 33% by the end of the study. The participants as a whole progressively decreased their calories by reducing all three macronutrients throughout the investigation. Thus, the two subjects uniquely increased their proportion of protein, possibly reducing fat and carbohydrate to the point of detriment. Related to this point, there was a correlation between FFM losses and declines in insulin and insulin-like growth factor 1 (IGF-1). The authors suggested that an increase in carbohydrate rather than protein in the final stages of this study may have offset these hormonal declines and subsequent FFM losses. While limited conclusions can be made from this study, it appears that increases in protein are only beneficial for ameliorating losses in FFM up to the point at which sufficient fat or carbohydrate levels are not compromised.

Of these six studies, only in Walberg et al., (1988) and Mettler et al., (2010) were different protein intakes compared to one another with well-matched groups and appropriate controls in place for diet, training and time spent in the intervention. While well-designed, Walberg et al., (1988) and Mettler et al., (2010) provide information on a total of only four protein intakes (0.8g/kg, 1g/kg, 1.6g/kg, and 2.3g/kg). While the time frame and range of protein intakes are limited, it seems that as protein is increased FFM retention increases as well.

In contrast, in a recent study not included in this analysis lasting three weeks, a non-significant trend of greater FFM retention was observed in a group consuming 1.6g/kg of protein compared to a group consuming 2.4g/kg (Pasiakos et al., 2013). However, the 2.4g/kg group consumed a diet that was 27% carbohydrate while the 1.6g/kg group consumed a diet that was 44% carbohydrate. The trend for greater FFM losses in the 2.4g/kg group may have

been related to decreases in insulin and IGF-1 (Maestu et al., 2010) or muscular endurance (Walberg et al., 1988). If muscular endurance was decreased in the higher protein group, it would have likely decreased performance considering the participants exclusively performed sets of 15 repetitions per exercise. Additionally, the training in this study was specifically designed to not provide an anabolic stimulus and only to maintain pre-study muscular fitness levels. This presents the possibility that if the training did not provide an anabolic stimulus, there may have not been an increased demand for protein. It is unknown whether the results would have been different had the participants in the higher protein group reduced their fat intake to allow for a greater amount of carbohydrate to be consumed. More importantly, this study's applications to resistance-trained athletes are limited since the participants were not required to be resistance-trained for inclusion and were not performing progressive strength training. However, the findings highlight the need for further study comparing high protein intakes with matched carbohydrate intakes.

It appears that FFM losses can be avoided only in populations with less resistance training experience of higher body fat when following slower weight loss regimens using current sports nutrition recommendations for protein intake (1.2-2.0g/kg). To date only Phillips and Van Loon (2011) have recommended higher intakes (1.8-2.7g/kg) for athletes during periods of negative energy balance and weight loss.

Conclusions and recommendations

The traditional protein recommendations for strength athletes have not been determined by examining athletes in a calorically-restricted state or at low body fat percentages and may be too low to minimise losses of FFM during these conditions. The recent recommendation by Phillips and Van Loon (2011) of consuming 1.8-2.7g/kg of protein is supported by the limited research available; however, to further customise protein intake within this range for the individual, the body composition of the athlete should be considered. Since protein recommendations are traditionally set based on the

study of individuals of a normal or high body fat percentage, it may be worthwhile to prescribe protein intake based on FFM versus total body mass in athletic populations. This may avoid giving recommendations that are too low for lean athletes.

When analysing the six studies reviewed to determine protein intake per kilogram of FFM, it appears that the range of 2.3-3.1g/kg of FFM is the most consistently protective intake against losses of lean tissue. Furthermore, the goal of the athlete should be taken into account. Athletes with a lower body fat percentage, or a primary goal of maintaining maximal FFM should aim towards the higher end of this range. Those who are not as lean, or who are concerned primarily with strength and performance versus maintenance of FFM can safely aim for the lower end of this recommendation.

It also appears that a reduction in dietary fat versus carbohydrate to create the bulk of the caloric deficit is more effective in maintaining performance. That said, too low of a fat intake could compromise health or well-being, thus a lower limit for fat intake of 20% of total calories is recommended. Furthermore, less extreme weight loss rates (0.5kg per week or 0.7% of total body mass) may serve an even more important role than protein intake in the preservation of FFM. Slower rates of weight loss appear to be more protective of both FFM and performance and will allow a greater "caloric budget" to assign values to the three macronutrients. Future research should measure the effects of varying protein intakes on FFM and performance in athletes of various sports, body compositions and macronutrient ratios for longer time periods than have been currently studied.

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CHAPTER 3 – PHYSIOLOGICAL EFFECTS OF PROTEIN MODIFICATION IN RESISTANCE-TRAINED MALES DURING CALORIC RESTRICTION: A DOUBLE-BLIND CROSSOVER STUDY

Summary

Objectives: Athletes risk performance and FFM loss when dieting. Dietary strategies to prevent losses are unclear. This study examined the effects of two diets on anthropometry, strength and stress in athletes. **Design:** This double-blind crossover included 13 resistance-trained males (20-43 yr). Methods: Participants followed carbohydrate-matched, high-protein low-fat (HPLF) or moderate-protein moderate-fat (MPMF) diets of 60% habitual calories for two weeks. Protein intakes were 2.8g/kg and 1.6g/kg and mean fat intakes were 15.4% and 36.5% of calories, respectively. Isometric midthigh pull (IMTP) and anthropometrics were measured at baseline and completion. The DALDA and Profile of Mood States (POMS) were completed daily. Outcomes were presented statistically as the probability of clinical benefit, triviality or harm with effect sizes for qualitative assessment. **Results:** Differences of effect between diets on IMTP and anthropometrics were likely or almost certainly trivial, respectively. Mood disturbance and stress occurred during both diets. "Worse than normal" scores on DALDA part A, part B and the part A "diet" item were likely more beneficial (effect sizes 0.32, 0.4 and 0.65, respectively) during HPLF relative to MPMF. The POMS fatigue score was likely more beneficial (effect size 0.37) and the POMS total mood disturbance score (TMDS) was possibly more beneficial (effect size 0.29) during HPLF relative to MPMF. Conclusions: For the two weeks observed, strength and anthropometric differences were minimal between diets while stress, fatigue and diet-dissatisfaction were substantially higher during MPMF. Therefore, a HPLF diet during short-term weight loss may be more effective at mitigating mood disturbance, fatigue, diet-dissatisfaction and stress than a MPMF diet.

Keywords: macronutrient, psychometric, anthropometry, resistance training, isometric, athlete.

Introduction

Caloric restriction during resistance training is common practice for athletes in weight-class restricted or aesthetic sport. This is frequently seen among bodybuilders, power lifters, weight lifters, wrestlers, and combat athletes (Mourier et al., 1997; Slater & Phillips, 2011; Umeda et al., 2004), the goal being to either make a weight-class division or improve performance. Performance improvement occurs either via increased power-to-weight ratio in performance sports, or via aesthetic improvement in physique competitions. These improvements should be maximised the larger the contribution of fat mass and the smaller the contribution of FFM is to weight loss. However, weight loss and caloric restriction can lead to losses of FFM and performance (Buford et al., 2006; Koral & Dosseville, 2009; Koutedakis et al., 1994; Layman, Boileau, et al., 2003; Umeda et al., 2004). Thus, strategies that try to minimise unfavourable changes in body composition and performance warrant study.

While it is well established that body composition is most favourably improved in overweight populations performing resistance training in combination with an increased protein intake (Demling & DeSanti, 2000; Layman et al., 2005; Stiegler & Cunliffe, 2006), similar research in resistance-trained athletes is sparse. To date, two studies exist in which resistance-trained athletes performing realistic training consumed energy-matched hypocaloric diets of differing protein levels and had performance and body composition measured (Mettler et al., 2010; Walberg et al., 1988). The data available from these studies provides insight into the FFM-sparing potential of high-protein diets in athletes, but only a handful of intake levels have been examined (0.8g/kg, 1g/kg, 1.6g/kg and 2.3g/kg per day). This small number of intakes limits the ability to determine thresholds at which benefits are obtained. However, it seems that as protein increases FFM retention increases as well.

In contrast, in Pasiakos et al., (2013) a non-significant trend of greater FFM retention was observed in a group consuming 1.6g/kg of protein compared to a group consuming 2.4g/kg. However, this trend could have

been related to a low carbohydrate-mediated (27% of calories) performance decrease in the resistance training protocol (Walberg et al., 1988), or because the protocol did not increase protein demands as it was specifically designed to not provide an anabolic stimulus. Thus, uncertainty remains for thresholds of beneficial protein intakes in resistance-trained athletes during weight loss.

To aid in the establishment of protein recommendations for resistance-trained athletes during weight loss, further study must be performed. Therefore, in the context of isocaloric, carbohydrate-matched diets of a 40% energy deficit, the current study compared 2.8g/kg to 1.6g/kg of protein per day; 2.8g/kg representing what many strength athletes habitually consume and 1.6g/kg falling within standard sports nutrition guidelines.

Methods

Thirteen resistance-trained males gave their written informed consent (see Appendix 3) to participate in this double-blind crossover study approved by the AUT University Ethics Committee (see Appendix 1). Participants were required to be: (i) regularly (≥ 2 days per week) resistance training with at least one year of experience; (ii) weight stable (± 2%) for at least one month; (iii) healthy as assessed by the Physical Activity Readiness Questionnaire (see Appendix 4); (iv) not using anabolic steroids or other illegal performance enhancing drugs; and (v) below 20% body fat (Durnin & Womersley, 1974) as assessed by an International Society for the Advancement of Kinanthropometry certified anthropometrist. Participants were recruited from gyms, weight lifting and crossfit clubs, supplement stores, and AUT University sports science and nutrition classes. Participant characteristics are provided in Table 4.

Table 4. Pre-intervention participant characteristics

Characteristic	HPLF	MPMF
n	13	13
Age (yr)	27.4 ± 7.9	27.4 ± 7.9
Height (cm)	177.9 ± 10.4	177.9 ± 10.4
Weight (kg)	80.6 ± 8.4	80.7 ± 8.0
BMI (kg/m²)	25.5 ± 1.7	25.5 ± 1.6
Sum of Eight Skinfolds (mm) ^a	71.7 ± 18.3	76.7 ± 21.0
Body Fat Percentage (%) ^b	13.2 ± 4.2	14.1 ± 3.7
FFM (kg) ^b	70.0 ± 8.0	69.3 ± 7.5

Values are means ± SD.

HPLF, high-protein low-fat; MPMF, moderate-protein moderate-fat; BMI, body mass index; FFM, fat free mass.

Eligible participants were taught to track their diets (see Appendix 8) and completed a one-week food diary that was analysed by the researchers, one of whom is a New Zealand Registered Dietitian, to determine habitual energy intakes. Digital food scales were provided to participants who did not have their own. Food preference questionnaires (see Appendix 5) were used to develop meal plans for each dietary intervention. Participants were not provided food, however each participant was provided with three meal plans with equal macronutrients, for variety, and either a pea-protein isolate (Clean Lean Protein Vanilla; NuZest, New Zealand) only or a pea-protein and maltodextrin mix to make up the daily intake during each intervention. Pea protein was selected to assist in blinding because of its thicker texture and infrequent commercial use compared to whey, casein or soy protein. Similarly, maltodextrin was selected because of its bland flavour. Participants were not permitted to consume any new supplements, but were instructed to maintain their existing supplementation regime throughout the two interventions. Details of the intervention diets are provided in Table 5.

^a Triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh and medial calf skinfolds.

^b Determined by the method of Durnin and Womersley (1974).

Table 5. Nutritional profiles of interventions

Intervention development	HPLF	MPMF		
Protein (g/kg)	2.8	1.6		
Carbohydrate (% of total calories)	35%	35%		
Fat	remaining	remaining		
Calories	60% habitual intake	60% habitual intake		
Total intakes (meal plans plus powders)	HPLF	MPMF		
Protein (g/day)	225.7 ± 24.5	129.0 ± 14.0		
Carbohydrate (g/day)	159.5 ± 21.9	159.5 ± 21.9		
Fat (g/day)	31.4 ± 14.2	74.2 ± 15.0		
Calories (kcal/day)	1829.3 ± 248.3	1829.3 ± 248.3		
Intakes from meal plans alone	HPLF	MPMF		
Protein (g/day)	115.3 ± 24.5	108.8 ± 14.0		
Carbohydrate (g/day)	151.8 ± 21.9	64.1 ± 21.9		
Fat (g/day)	29.7 ± 14.2	73.9 ± 15.0		
Calories (kcal/day)	1341.6 ± 248.3	1364.0 ± 248.3		
Intakes from supplement powders alone	HPLF	MPMF		
Protein (g/day)	110.4	20.2		
Carbohydrate (g/day)	7.7	95.4		
Fat (g/day)	1.7	0.3		
Calories (kcal/day)	487.7	465.3		

Values are means ± SD when applicable.

HPLF, high-protein low-fat; MPMF, moderate-protein moderate-fat.

The dietitian, who was not involved in data collection or analysis mixed the powders, labelled them "a" or "b" for blinding and retained the blinding key. The primary researcher was un-blinded to the supplement key only after data analysis was complete. The participants were instructed to approach the dietitian for any assistance with dietary matters.

Three participants inadvertently consumed extra or incorrect food items on one occasion each, and immediately communicated this with the dietitian. Meals for the remainder of the day were adjusted where possible to ensure that the macronutrient and energy contributions were not compromised. When the communication was made the day following the error, meals on that day were adjusted to account for the nutrient shortfall or excess the day prior, and thereby ensuring an accurate nutrient intake over a two-day period. Examples of errors included inadvertently adding milk to tea, consumption of a full-sugar beverage and full-sugar peppermints, rather than

their non-sugar counterparts. All meal plans were successfully adjusted, thereby maintaining nutrient integrity throughout the study.

Participants were randomly assigned to either the HPLF or MPMF diet resulting in six of the participants beginning with HPLF and finishing with MPMF and seven having the opposite order. After completing the first diet, participants were assigned to a wash out that lasted approximately twice the length of the intervention (two weeks) or longer (25 to 49 days) based on the scheduling needs of the participant. During the wash out participants were instructed to eat normally, initially allowing weight regain. Two weeks before starting the second intervention they were instructed to return to their habitual caloric intake as recorded prior to beginning their first intervention. Fourteen participants started the study; however, one dropped out during the first intervention complaining of fatigue, depression and mental stress.

Before and the day after each intervention, participants had their height, weight, eight-site (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, and medial calf) skinfold thicknesses, waist, hip, calf, relaxed and flexed arm girths, and femur and humerus breadths measured. The anthropometrist had a technical error of measurement for skinfolds of \pm 0.4mm (mean error of six sites). Three equations were used to analyse FFM (Durnin & Womersley, 1974; M. J. Peterson, Czerwinski, & Siervogel, 2003; Yuhasz, 1974).

Pre and post testing occurred as close to the same time of day as possible for each individual. Additionally, the same exercise and dietary regime was maintained prior to both pre and post testing. Strength assessment testing peak force using the IMTP exercise (Haff et al., 1997; Stone et al., 2003) followed anthropometry. Vertical ground reaction force data were collected at a sample rate of 200 Hz with a commercially available force plate (400 Series Performance Force Plate; FitnessTechnology, Australia) connected to computer software (Ballistic Measurement System, FitnessTechnology, Australia). The force plate was calibrated before each testing session. Participants used cloth lifting straps to avoid grip-strength limitations. One participant during one pre test was unable to properly use

the lifting straps and was excluded from the IMTP analysis for that arm of the crossover.

The POMS short form (Shacham, 1983) and the DALDA (Rushall, 1990) questionnaires (see Appendices 6 and 7, respectively) were chosen to quantify the stress-response to the diets because of their wide use in sport and exercise research (Halson et al., 2002; Mettler et al., 2010; Morgan, Costill, Flynn, Raglin, & O'Connor, 1988; Nicholls, Backhouse, Polman, & McKenna, 2009). Every day participants completed the POMS and both part A and B of the DALDA questionnaire, which represent the general stress sources and the resulting signs and symptoms, respectively. Each source of stress in part A and each sign or symptom in part B could be graded as follow: a = "worse than normal", b = "normal" and c = "better than normal". Total "worse than normal" scores were calculated for part A and part B and total "worse than normal" scores for the "diet" item in part A were calculated to assess the dietrelated stress, representing the satiety and enjoyment of each dietary intervention. If a participant missed a day of completing the DALDA in one 14day intervention, the same day of the following 14-day intervention was discarded. The POMS questionnaire provides a measure of the tension/anxiety, depression, anger, vigour, fatigue and confusion levels of the participant. Each mood item in the POMS was rated on a 5-point scale as follows: 0 = "not at all", 1 = "a little", 2 = "moderately", 3 = "quite a bit" and 4 = "extremely". For each participant a mean of all days reported was calculated for the TMDS and fatigue score. TMDS was calculated by adding the five negative mood states together and subtracting the positive mood state, vigour. One participant after one intervention lost their POMS and DALDA forms and thus their data for that intervention period was not included.

All variables except the POMS TMDS, fatigue score and the DALDA "worse than normal" diet scores were log transformed before analysis to reduce non-uniformity of error and to express effects as percent changes (Hopkins et al., 2009). POMS TMDS and fatigue scores were averaged over the days reported to provide uniformity and the DALDA diet score is a single item Likert scale.

A spreadsheet for analysis of a post-only crossover trial (Hopkins, 2012a) was used to determine differences between the two groups on the POMS and DALDA scores. A spreadsheet for analysis of a pre and post crossover trial (Hopkins, 2012b) was used to determine differences between the two groups on the IMTP and all anthropometric variables. IMTP data were analysed with bodyweight at the start of the intervention as a covariate. Mean percentage changes with 90% confidence limits (CL) were presented for pre and post test variables (IMTP and anthropometric data) and mean scores with 90% CL were presented for post-only variables (psychometric data). The chances (% and qualitative) that the true value of each statistic was practically beneficial, trivial, or harmful were calculated using the spreadsheets. To determine the threshold for an effect, the smallest standardised change was assumed to be 0.2. For all variables that were measured, thresholds used to determine the magnitude of effect sizes were based on a modified Cohen's scale. Standardised thresholds of <0.2, <0.6, <1.2, and <2.0 were interpreted as trivial, small, moderate, and large effects, respectively (Batterham & Hopkins, 2006). This approach using probability statistics allows the reader to make decisions around the use of feedback based on its predicted beneficial or harmful effects (Batterham & Hopkins, 2006; Hopkins et al., 2009).

Results

Results of all variables measured are provided in Table 6.

Table 6. Effect of different energy restriction diets on anthropometric, performance and psychometric outcomes in resistance trained athletes

Measure	HPLF mean change ± 90% CL (%)	MPMF mean change ± 90% CL (%)	Chances that true effect of HPLF relative to MPMF are substantially			Qualitative assessment of HPLF's effect relative to
			Harmful (%)	Trivial (%)	Beneficial (%)	MPMF 's effect
Anthropometric						
Bodyweight	-3.6 ± 0.6	-4.2 ± 0.6	<0.1	99	<0.1	Almost certainly trivial
Body fat, sum of eight skinfolds	-12.5 ± 3.4	-12.6 ± 3.5	1	97	2	Very likely trivial
FFM (Durnin & Womersley, 1974)	-2.1 ± 0.7	-2.5 ± 0.6	<0.1	99	<0.1	Almost certainly trivial
FFM (Yuhasz, 1974)	-2.9 ± 0.6	-3.3 ± 0.6	<0.1	99	<0.1	Almost certainly trivial
FFM (M. J. Peterson et al., 2003)	-2.1 ± 0.6	-2.5 ± 0.6	<0.1	99	<0.1	Almost certainly trivial
Performance						
IMTP	-2.5 ± 1.4	-0.4 ± 3.5	19	81	<0.1	Likely trivial
Psychometric	HPLF mean ± 90% CL	MPMF mean ± 90% CL				
DALDA "worse than normal" scoring	-	-	-	-	-	-
Part A total	16.7 ± 6.3	28.8 ± 10.8	<0.1	17	83	Likely beneficial
Part B total	37.3 ± 16.7	66.0 ± 22.2	<0.1	14	86	Likely beneficial
Part A "diet"	4.9 ± 1.9	7.5 ± 2.1	<0.1	5	95	Likely beneficial
POMS average TMDS	0.7 ± 4.1	6.5 ± 6.5	<0.1	25	75	Possibly beneficial
POMS average fatigue score	2.8 ± 1.5	4.7 ± 2.1	1	18	81	Likely beneficial

HPLF, high-protein low-fat; MPMF, moderate-protein moderate-fat; CL, confidence limits; FFM, fat free mass; IMTP, isometric mid-thigh pull; DALDA, daily analysis of life demands of athletes; POMS, profile of mood states; TMDS, total mood disturbance score

Qualitative assessment was determined as follows: <1%, almost certainly not; 1-5%, very unlikely; 5-25% unlikely; 25-75% possibly; 75-95%, likely, 95-99%, very likely; >99%, almost certainly.

All anthropometric markers decreased in both groups over the course of the two-week intervention. Fat loss as measured by sum of eight skinfolds was almost identical between interventions. A slightly greater amount of bodyweight (0.6%) and FFM (0.4%) as assessed by all three equations was lost in the MPMF group. However, all anthropometric differences between diets were very likely or almost certainly trivial.

IMTP strength losses were slightly less (1.1%) for the MPMF group but more than twice as variable as the HPLF group. However, differences in effect on isometric strength between diets were likely trivial.

Mood disturbance and stress occurred during both diets. General stress measured by part A of the DALDA, signs and symptoms of stress measured by part B of the DALDA and the part A "diet" item stress levels were higher in MPMF compared to HPLF. The effect sizes for these differences were small for part A (0.32) and B (0.4) of the DALDA and moderate for the part A "diet" item (0.65). POMS fatigue and TMDS were higher in the MPMF groups as well with small effect sizes (0.37 and 0.29, respectively) for these differences. Effects of HPLF relative to MPMF on all parts of the DALDA and POMS fatigue were assessed as likely beneficial, and the effect on TMDS was assessed as possibly beneficial. The amount of dietary carbohydrate coming from maltodextrin were analysed as a covariate separately to determine if these effects were related to carbohydrate source rather than amount; however, this analysis did not change the qualitative outcomes.

Discussion

From the findings it is suggested that during short-term (2 weeks) periods of substantial (40% reduction) energy restriction of different macronutrient composition, a high-protein (2.8g/kg) low-fat (mean 15.4% of calories) approach provides lower ratings of athlete-specific stress, fatigue, mood disturbance and diet dissatisfaction than a moderate-protein (1.6g/kg) moderate-fat (mean 36.5% of calories) approach. The finding of diet dissatisfaction being higher during MPMF is novel, because even though protein's satiating effect is documented (Leidy, Armstrong, Tang, Mattes, &

Campbell, 2010), rarely is it compared directly with fat. Rather, comparisons are typically made between carbohydrate and fat (Cotton, Burley, Weststrate, & Blundell, 2007). However, the psychometric findings cannot be attributed to satiety alone. The sole question related to nutrition appears on DALDA part A, while DALDA part B and the POMS have no questions related to diet, nutrition or hunger.

Fatigue sub-scale scores, TMDS and DALDA part B results indicate that athlete-specific stress and fatigue were meaningfully higher during MPMF. Potential causes for these findings were that the higher protein intake during HPLF may have helped to stabilise blood glucose levels (Layman, Shiue, Sather, Erickson, & Baum, 2003), or because at high protein intakes as much as 60% of endogenous glucose production comes from gluconeogenesis (Bilsborough & Mann, 2006; Linn et al., 2000). Higher levels of glycogen and increased hepatic glucose production could reduce athlete-specific ratings of stress and fatigue. Finally, it cannot be ruled out that a loss of FFM (to include muscle mass or glycogen) may have contributed to the psychometric results. With only a two-week time window and comparing diets at and above protein recommendations for athletes, it is not a surprise the anthropometric differences were small.

While anthropometric equations do not provide enough precision to confidently make statements about FFM changes, body mass and skinfold thickness when not used in an equation are much more reliable measures (± 0.4mm technical error of measurement in this study). The energy content of muscle and glycogen is 50% and 45% that of body fat, respectively, at equated masses (Hall, 2007b). Therefore, at identical caloric deficits, weight loss will be greater if a larger percentage of energy contribution to weight loss originates from FFM. The fact that changes in skinfolds were practically identical while body mass decreased by 0.6% (0.4kg) more during MPMF than HPLF, may indicate this additional loss of mass could have originated from FFM. While deemed trivial for this two-week period, these results may not have remained trivial had the intervention continued for a longer period and could have been

a contributing factor to the increased ratings of athlete-specific stress and fatigue.

Worthy of note, while the differences in peak force production between diets were likely trivial, there is a 19% chance that MPMF might prove to be a more beneficial approach to maintenance of peak force than HPLF, while there is practically no chance of the opposite. Intra-muscular fatty acid levels are replenished to a much lesser degree when consuming 15% of calories from fat compared to 40% of calories from fat (Boesch, Kreis, Hoppeler, Decombaz, & Fleith, 2000) and despite common perception that carbohydrate alone fuels resistance training, intra-muscular triglyceride does contribute to energy expended during heavy resistance exercise of relatively short duration in men (Essen-Gustavsson & Tesch, 1990). Thus, it is possible that the low fat intake of 15% of calories in HPLF may have impacted training in some of the participants in such a way that IMTP peak force was negatively affected. In a practical sense, this can be alleviated with slower weight loss which not only appears to be better for performance maintenance (Mero et al., 2010), but would also allow for a greater energy contribution from fat while keeping protein intake high.

Finally, it should be noted that mean effects can mask individual responses. A number of the participants responded much more favourably to one diet compared to the other. One participant had an 11% increase in their peak force after following the MPMF diet and a 1% increase after the HPLF diet. Another experienced an 11% reduction in their peak force following the MPMF diet and a 4% reduction after the HPLF diet. Likewise one participant had a twofold greater decrease in their sum of eight skinfolds after the HPLF diet compared to the MPMF intervention while another participant experienced the exact opposite. Additionally, individual body composition appeared to affect response. There were striking differences between the participants lowest in body fat compared to the rest. The two leanest participants began both interventions with a sum of eight skinfolds between 36 and 45 mm. These two participants reported the first, second or third highest levels of stress, fatigue and mood disturbance among all participants.

Conclusion

Differences between protein intakes in the range of 1.6-2.8g/kg and fat intakes between 15.4-36.5% of calories on body composition and maximal strength during short-term weight loss are likely inconsequential. However, higher than typically recommended protein intakes may mitigate fatigue and stress which would likely increase dietary adherence for longer term caloric restriction. Practitioners should take into account the influence of body composition and the individual responses to varying macronutrient ratios when developing nutrition plans for athletes during caloric restriction. Much remains in question and research utilising reliable measures of body composition is required. But, arguably the largest gap in the current research is the study of long-term energy restriction in athletes. Considering the adaptability of human physiology, longer periods of energy restriction may have significantly different ramifications than short-term diets and warrant study.

Practical implications:

- Variations between 1.6g/kg and 2.8g/kg of protein and 15% and 35% of calories from fat during energy restriction will most likely have negligible impacts on maximal strength and body composition in the short term.
- Increasing protein intake above typically recommended sports nutrition guidelines during energy restriction may confer benefits to stress and fatigue reduction. For longer term diets these effects may enhance adherence.
- Smaller caloric deficits (70-80% of habitual calories) than represented in this study (60%) are recommended. Higher caloric intakes and slower rates of weight loss that allow a more balanced intake of macronutrients may maintain performance more consistently.

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CHAPTER 4 - DISCUSSION AND PRACTICAL APPLICATION

Preface

This chapter is a synthesis of the findings of this thesis with practical application, in context with the current body of knowledge, to inform nutritional practice for competing bodybuilders. This chapter closes with a conclusion of the thesis as a whole. Excerpts from this chapter appear in the article "Evidence-based recommendations for natural bodybuilding: Nutrition and supplementation" which was recently submitted to the Journal of the International Society of Sports Nutrition. The submitted article is a narrative review that contains recommendations for nutrient timing, supplementation, psychosocial issues and contest peaking not included here, authored by myself and two co-authors.

Introduction:

Unlike athletes whose sports include aesthetic elements in their judging, bodybuilders are judged exclusively in a qualitative manner on the display of lean, proportionate muscularity. To achieve competitive success they undergo prolonged, rigorous diets which take them to minimal body fat levels, all the while attempting to maintain muscle mass with resistance training. They straddle the often opposing concepts of performance and weight-loss nutrition. Therefore, careful attention to caloric and macronutrient intake is required to maintain performance while losing maximal body fat.

Since any macronutrient alteration in the context of a fixed caloric intake necessitates a shift in another, this chapter provides evidence-based guidelines on how to first establish caloric intake for competition, and then determine the macronutrient contributions to calories consumed.

Caloric intake for competition

To create weight loss, more energy must be expended than consumed.

This can be accomplished by increasing caloric expenditure while reducing

caloric intake. The size of this caloric deficit and the length of time it is maintained will determine how much weight is lost. Every pound of pure body fat that is metabolised yields approximately 3500 kcals, thus a daily caloric deficit of 500 kcals theoretically results in fat loss of approximately half a kilogram per week if the weight loss comes entirely from body fat (Hall, 2007b). However, the tissue lost during the course of an energy deficit is influenced by the size of the energy deficit. While greater deficits yield faster weight loss, the percentage of weight loss coming from FFM tends to increase as the size of the deficit increases (Forbes, 2000; Garthe, Raastad, Refsnes, et al., 2011; Hall, 2007a, 2007b).

In studies of weight loss rates, weekly losses of 1kg over 4 weeks resulted in a 30% greater reduction in testosterone levels and a 5% decrease in bench press strength compared to no decrease in strength training women losing 0.5kg weekly (Mero et al., 2010). Weekly weight loss rates of 1.4% of bodyweight compared to 0.7% in athletes during caloric restriction lasting four to eleven weeks resulted in reductions of fat mass of 21% in the faster weight loss group and 31% in the slower loss group. In addition, FFM increased on average by 2.1% in the slower loss group while remaining unchanged in the faster loss group. Worthy of note, small amounts of FFM were lost among leaner subjects in the faster loss group (Garthe, Raastad, Refsnes, et al., 2011).

Therefore, weight loss rates that are more gradual may be superior for FFM retention. At a loss rate of 0.5kg per week (assuming a majority of weight lost is fat mass), a 70kg athlete at 13% body fat would need to be no more than 6kg to 7kg over their contest weight in order to achieve the lowest body fat percentages recorded in competitive bodybuilders following a traditional three-month preparation (Bamman, Hunter, Newton, Roney, & Khaled, 1993; Maestu et al., 2010; Sandoval, Heyward, & Lyons, 1989; van der Ploeg et al., 2001; Walberg-Rankin, Edmonds, & Gwazdauskas, 1993; Withers et al., 1997). If a competitor is not this lean at the start of the preparation, faster weight loss will be required which may carry a greater risk for FFM loss.

In a study of bodybuilders during the 12 weeks before competition, male competitors reduced their caloric intake significantly during the latter half and subsequently lost the greatest amount of FFM in the final three weeks (Newton, Hunter, Bammon, & Roney, 1993). Therefore, diets longer than two to four months with less severe caloric deficits (approximately 0.5kg or 0.7% of bodyweight weekly), may be superior for FFM retention compared to shorter or more aggressive diets. Ample time should be allotted to lose body fat to avoid an aggressive deficit and the length of preparation should be tailored to the competitor, with leaner athletes dieting for shorter periods than those with higher body fat percentages. It must also be taken into consideration that the leaner the competitor becomes the greater the risk for FFM loss. As the availability of adipose tissue declines the likelihood of muscle loss increases (Forbes, 2000; Hall, 2007a), thus it may be best to pursue a more gradual approach to weight loss towards the end of the preparation diet compared to the beginning to avoid FFM loss.

Determining macronutrient intake

Protein

Adequate protein consumption during contest preparation is required to support maintenance of FFM. Athletes require higher protein intakes to support increased activity and strength athletes benefit from higher intakes to support growth of FFM (Butterfield, 1987; Lambert, Frank, & Evans, 2004; Lemon, 2000; Phillips, 2006; Phillips et al., 2007; Phillips & Van Loon, 2011; Slater & Phillips, 2011; Tipton & Wolfe, 2004). Some researchers suggest these requirements increase further when athletes undergo energy restriction (Butterfield, 1987; Garthe, Raastad, Refsnes, et al., 2011; Mero et al., 2010; Mettler et al., 2010; Millward, 2004; Phillips & Van Loon, 2011; Stiegler & Cunliffe, 2006; Walberg et al., 1988). Furthermore, there is evidence that protein requirements are higher for leaner individuals in comparison to those with higher body fat percentages (Elia et al., 1999; Hall, 2007b).

The collective agreement among reviewers is that a protein intake of 1.2-2.2g/kg is sufficient to allow adaptation to training for athletes with

average body compositions whom are at or above their energy needs (Lemon, 2000; Phillips, 2004, 2006; Phillips et al., 2007; Phillips & Van Loon, 2011; Slater & Phillips, 2011; Tarnopolsky, 2008; Tipton, 2008; Tipton & Wolfe, 2004; Wilson & Wilson, 2006). However, bodybuilders during their contest preparation period typically perform resistance and cardiovascular training, restrict calories and achieve very lean conditions (Bamman et al., 1993; Kleiner, Bazzarre, & Litchford, 1990; Lambert et al., 2004; Maestu et al., 2010; Newton et al., 1993; Sandoval & Heyward, 1991; Sandoval et al., 1989; van der Ploeg et al., 2001; Walberg-Rankin et al., 1993; Withers et al., 1997). Each of these factors increases protein requirements and when these factors are compounded, protein needs may increase further. Therefore, optimal protein intakes for bodybuilders during contest preparation may be significantly higher than existing recommendations.

In the only two controlled studies on resistance-trained athletes during caloric restriction most representative of bodybuilders, it can be concluded that linear increases in protein intake from 0.8-2.3g/kg increase FFM retention (Mettler et al., 2010; Walberg et al., 1988). Only the observations of Pasiakos et al., (2013) are in contrast with this conclusion, in which 2.4g/kg of protein did not confer greater FFM protection than 1.6g/kg. However, these results may have been due to the low carbohydrate intake (121g \pm 8) of the 2.4g/kg group or because the population studied was not resistance-trained or performing real-world progressive resistance training likely to increase protein needs.

Therefore, as previously discussed in Chapter 2, based on the limited data available a protein intake range of 1.8-2.7g/kg (Phillips & Van Loon, 2011) of total body mass or 2.3-3.1g/kg of FFM is likely optimal for FFM retention in lean, energy-restricted, resistance-trained athletes. Within this range, lower body fat levels are a proposed reason to increase protein intake (Elia et al., 1999) as well as having the primary goal of FFM preservation rather than performance maintenance; the latter of which might benefit from a lower protein intake in favour of increased carbohydrate or fat. Furthermore, based on the findings of Chapter 3, less fatigue, mood

disturbance, diet dissatisfaction and athlete-specific stress may be experienced while consuming protein near the upper, rather than the lower end of this range. Thus, bodybuilders most likely should consume an intake at the higher end of the proposed ranges of 1.8-2.7g/kg of total body mass or 2.3-3.1g/kg of FFM.

Carbohydrate

Current sports nutrition guidelines for high intensity activities recommend an individualised approach to carbohydrate intake to ensure carbohydrate availability ("IOC consensus statement on sports nutrition 2010," 2011). Inadequate carbohydrate can impair strength training (Leveritt & Abernethy, 1999) and consuming adequate carbohydrate prior to training can reduce glycogen depletion (Haff et al., 2000) and may therefore enhance performance.

While it is true that resistance training utilises glycogen as its main fuel source (MacDougall et al., 1999), total caloric expenditure of strength athletes is less than that of mixed sport and endurance athletes. Thus, authors of a review recommend that carbohydrate intakes for strength sports, including bodybuilding, be between 4g/kg and 7g/kg depending on the phase of training (Slater & Phillips, 2011). However, in the specific case of a bodybuilder in contest preparation, achieving the necessary caloric deficit while consuming adequate protein and fat would likely not allow consumption at the higher end of this recommendation.

Satiety and fat loss generally improve with lower carbohydrate diets; specifically with higher protein to carbohydrate ratios (Halton & Hu, 2004; Layman & Baum, 2004; Layman, Boileau, et al., 2003; Smeets, Soenen, Luscombe-Marsh, Ueland, & Westerterp-Plantenga, 2008; Veldhorst et al., 2008; Westerterp-Plantenga, 2008). In terms of performance and health, low carbohydrate diets are not necessarily as detrimental as typically espoused (Cook & Haub, 2007). In a recent review, it was recommended for strength athletes training in a calorically-restricted state to reduce carbohydrate content while increasing protein to maximise fat oxidation and preserve FFM

(Phillips & Van Loon, 2011). However, the optimal reduction of carbohydrate and point at which carbohydrate reduction becomes detrimental likely needs to be determined individually.

One comparison of two isocaloric, energy-restricted diets in bodybuilders showed that a diet that provided adequate carbohydrate at the expense of protein (1g/kg) resulted in greater FFM losses compared to a diet that increased protein (1.6g/kg) through a reduction of carbohydrate (Walberg et al., 1988). However, muscular endurance was degraded in the lower carbohydrate group. In a study of athletes taking in the same amount of protein (1.6g/kg) during weight loss, performance decrements and FFM losses were avoided when adequate carbohydrate was maintained and dietary fat was lowered (Garthe, Raastad, Refsnes, et al., 2011). Mettler, et al., (2010) also found that a caloric reduction coming from dietary fat while maintaining adequate carbohydrate intake and increasing protein to 2.3g/kg maintained performance and almost completely eliminated FFM losses in resistancetrained subjects. Finally, in Pasiakos et al., (2013) participants undergoing an equal calorie deficit and consuming the same amount of protein as those observed in Mettler et al., (2010) lost three times the amount of FFM over the same time period (0.9kg in the first two weeks of energy restriction versus 0.3kg). One key difference was the highest protein group in Mettler et al., (2010) consumed a dietary carbohydrate intake equal to 51% of energy while the comparable group in Pasiakos et al., (Pasiakos et al., 2013) consumed 27% of their energy from dietary carbohydrate. While performance was not measured, the participants in Pasiakos et al., (2013) performing sets exclusively of 15 repetitions very likely would have experienced decrements in performance (Walberg et al., 1988) which could have been one component that lead to the greater losses of FFM.

While it appears low carbohydrate, high-protein diets can be effective for weight loss, a practical carbohydrate threshold appears to exist where further reductions negatively impact performance and risk FFM losses. In support of this notion, researchers studying bodybuilders during the final stages of contest preparation concluded that increasing carbohydrate during

the final phases of their diet may be a viable strategy to mitigate metabolic and hormonal adaptations that are associated with reductions in FFM (Maestu et al., 2010).

Fat

The importance of carbohydrate and protein in sports nutrition is often emphasised over that of dietary fat. Subsequently, recommendations typically focus on maintaining adequate fat intake while emphasising carbohydrate to fuel performance and protein to build and repair FFM. However, there is evidence that dietary fat influences anabolic hormone concentrations which may be of interest to bodybuilders attempting to maintain FFM while dieting (Lambert et al., 2004; Sallinen et al., 2004; Slater & Phillips, 2011; Volek, Kraemer, Bush, Incledon, & Boetes, 1997).

Reductions in the percentage of dietary fat in isocaloric diets from approximately 40% to 20% has resulted in modest, but significant, reductions in testosterone levels (Dorgan et al., 1996; Hämäläinen, Adlercreutz, Puska, & Pietinen, 1983). However, distinguishing the effects of reducing total dietary fat on hormonal levels from changes in caloric intake and percentages of saturated and unsaturated fatty acids in the diet is difficult (Hämäläinen, Adlercreutz, Puska, & Pietinen, 1984; Sallinen et al., 2004; Volek et al., 1997). In a study by Volek et al., (1997), correlations were found between testosterone levels, macronutrient ratios, types of lipids, and total dietary fat, illustrating a complex interaction of variables. In a similar study with resistance-trained males, correlations were found between testosterone, protein, fat and saturated fat which lead the researchers to conclude that diets too low in fat or too high in protein might impair the hormonal response to training (Sallinen et al., 2004). However, acute hormonal responses to training may not be correlated to actual changes in muscle mass (West & Phillips, 2011).

Competing bodybuilders must make an obligatory caloric reduction. If a reduction in fat is utilised, it may be possible to attenuate a drop in testosterone by maintaining adequate consumption of saturated fat (Lambert et al., 2004). However, a drop in testosterone does not equate to a reduction in FFM. In direct studies of resistance-trained athletes undergoing calorically-restricted high-protein diets, low fat interventions that maintain carbohydrate levels (Garthe, Raastad, Refsnes, et al., 2011; Mettler et al., 2010) appear to be more effective at preventing FFM loses than lower carbohydrate, higher fat approaches (Pasiakos et al., 2013; Walberg et al., 1988). This might indicate that attempting to maintain resistance training performance with higher carbohydrate intakes is more effective for FFM retention than attempting to maintain testosterone levels with higher fat intakes.

Body composition and caloric restriction may play greater roles in influencing testosterone levels than fat intake. During starvation, a reduction in testosterone occurs in normal weight, but not obese males (Suryanarayana et al., 1969). In addition, rate of weight loss may influence testosterone levels. Weekly target weight loss rates of 1kg resulted in a 30% reduction in testosterone compared to target weight loss rates of 0.5kg per week in resistance-trained women of normal weight (Mero et al., 2010). Additionally, an initial drop in testosterone occurred in the first six weeks of contest preparation in a group of drug-free bodybuilders despite various macronutrient percentages (Maestu et al., 2010). In a one-year case study of a natural competitive bodybuilder testosterone levels fell to one-fourth their baseline values three months into the six-month preparation period, then fully recovered three months into the six-month recovery period. Testosterone levels did not decline further after the initial drop at the threemonth mark despite a slight decrease in fat intake from 27% to 25% of calories at the six-month mark. Furthermore, the quadrupling of testosterone during the recovery period from its suppressed state back to baseline was accompanied by only a minor increase in calories from fat (percentage of calories from fat during recovery was between 30 and 35%) (Rossow, Fukuda, Fahs, Loenneke, & Stout, 2013). Thus, these testosterone changes in men appear mostly related to energy availability (body fat content and energy balance), and not surprisingly low levels of sustained energy availability are also the proposed cause of the hormonal disturbance "athletic amenorrhea"

in women (Loucks, Verdun, & Heath, 1998). Therefore, based on the collective data it appears that when extremely lean body compositions are attained through extended, relatively aggressive dieting, the caloric deficit and loss of body fat itself may have a greater impact on testosterone than the percentage of calories coming from dietary fat.

While cogent arguments for fat intakes between 20% and 30% of calories have been made to optimise testosterone levels in strength athletes (Bird, 2010), in some cases this intake may be unrealistic in the context of caloric restriction without compromising sufficient protein or carbohydrate intakes. While dieting, low carbohydrate diets may degrade performance (Walberg et al., 1988) and lead to lowered insulin and IGF-1 which appear to be more closely correlated to FFM preservation than testosterone (Maestu et al., 2010). Thus, fat intakes between 15% and 20% of calories, which have been previously recommended for bodybuilders (Lambert et al., 2004), can be deemed appropriate if higher percentages would reduce carbohydrate or protein below ideal ranges.

Macronutrient recommendations:

After caloric intake is established based on the time frame before competition (Turocy et al., 2011), body composition of the athlete (Elia et al., 1999; Forbes, 2000; Hall, 2007a), and keeping the deficit modest to avoid FFM losses (Garthe, Raastad, Refsnes, et al., 2011; Mero et al., 2010), macronutrients can be determined within this caloric allotment. A protein intake within the range of 1.8-2.7g/kg (Phillips & Van Loon, 2011) or 2.3-3.1g/kg of FFM should be set and scaled up within this range if the athlete has a leaner body composition and when or if a higher volume of training is prescribed. Fat intake should be in the range of 15-30% (Bird, 2010; Lambert et al., 2004) of calories, and remaining calories should come from carbohydrate. If training performance degrades it may prove beneficial to decrease the percentage of calories from dietary fat within these ranges in favour of a greater proportion of carbohydrate.

Conclusion:

Weight class-restricted and aesthetic athletes have unique requirements related not only to the metabolic and adaptive demands of their sport and training, but also related to their body composition and the stress and psychological demands of dieting. While traditionally recommended protein intakes are likely appropriate for athletes during energy balance or surplus, there are potential benefits to exceeding this amount during periods of energy deficit. Lean individuals experienced with resistance training may undergo reduced losses of FFM when they consume higher protein intakes based on the findings in Chapter 2. Furthermore, Chapter 3 highlights the potential benefits of reduced mood disturbance, fatigue and athlete-specific stress when consuming protein intakes above traditionally recommended levels during caloric restriction.

However, energy balance itself is likely a more important factor than protein intake alone. Slower weight loss rates and more moderate caloric restriction protects against performance and FFM losses. A gradual approach to weight loss rather than an aggressive one can be a more effective strategy for FFM and performance retention given ample planning and time. Care should be taken to ensure that adequate calories are consumed during periods of energy restriction. This ensures that when protein is increased, neither fat nor carbohydrate is driven too low.

Future research is required to determine how the effects of higher protein intakes impact body composition, performance and psychometric variables in lean resistance-trained athletes for longer time periods than have yet been studied. Questions also remain as to the effects of other macronutrient combinations and levels of caloric restriction. Finally, the majority of available studies in this area examine men; similar research in female athletes is severely lacking and needed.

REFERENCES

- Aragon, A. A., & Schoenfeld, B. J. (2013). Nutrient timing revisited: is there a post-exercise anabolic window? *Journal of the International Society of Sports Nutrition*, 10(1), 5.
- Ayvaz, G., & Çimen, A. R. (2011). Methods for body composition analysis in adults. *Open Obesity Journal*, *3*, 57-64.
- Bamman, M. M., Hunter, G. R., Newton, L. E., Roney, R. K., & Khaled, M. A. (1993). Changes in body composition, diet, and strength of bodybuilders during the 12 weeks prior to competition. *Journal of Sports Medicine and Physical Fitness*, *33*(4), 383-391.
- Batterham, A. M., & Hopkins, W. G. (2006). Making meaningful inferences about magnitudes. *International journal of sports physiology and performance*, 1(1), 50-57.
- Bilsborough, S., & Mann, N. (2006). A review of issues of dietary protein intake in humans. *International Journal of Sport Nutrition and Exercise*Metabolism, 16(2), 129.
- Bird, S. P. (2010). Strength nutrition: Maximizing your anabolic potential. Strength and Conditioning Journal, 32(4), 80-86.
- Boesch, C., Kreis, R., Hoppeler, H., Decombaz, J., & Fleith, M. (2000). Effect of diet on the replenishment of intramyocellular lipids after exercise. *European Journal of Nutrition, 39*(6), 244.
- Brughelli, M., Cronin, J., Levin, G., & Chaouachi, A. (2008). Understanding change of direction ability in sport: a review of resistance training studies. *Sports Medicine*, *38*(12), 1045-1063.
- Buford, T. W., Rossi, S. J., Smith, D. B., O'Brien, M. S., & Pickering, C. (2006).

 The effect of a competitive wrestling season on body weight,
 hydration, and muscular performance in collegiate wrestlers. *Journal*of Strength and Conditioning Research, 20(3), 689-692.
- Butterfield, G. E. (1987). Whole-body protein utilization in humans. *Medicine* and Science in Sports and Exercise, 19(5 Suppl), S157-165.

- Celejowa, I., & Homa, M. (1970). Food intake, nitrogen and energy balance in Polish weight lifters, during a training camp. *Nutrition and Metabolism*, 12(5), 259-274.
- Chaston, T. B., Dixon, J. B., & O'Brien, P. E. (2007). Changes in fat-free mass during significant weight loss: a systematic review. *International Journal of Obesity*, *31*(5), 743-750.
- Cook, C. M., & Haub, M. D. (2007). Low-carbohydrate diets and performance.

 *Current Sports Medicine Reports, 6(4), 225-229.
- Cotton, J. R., Burley, V. J., Weststrate, J. A., & Blundell, J. E. (2007). Dietary fat and appetite: similarities and differences in the satiating effect of meals supplemented with either fat or carbohydrate. *Journal of Human Nutrition and Dietetics*, 20(3), 186-199.
- Demling, R. H., & DeSanti, L. (2000). Effect of a hypocaloric diet, increased protein intake and resistance training on lean mass gains and fat mass loss in overweight police officers. *Annals of Nutrition and Metabolism*, *44*(1), 21-29.
- Dorgan, J. F., Judd, J. T., Longcope, C., Brown, C., Schatzkin, A., Clevidence, B.

 A., . . . Taylor, P. R. (1996). Effects of dietary fat and fiber on plasma
 and urine androgens and estrogens in men: a controlled feeding study. *American Journal of Clinical Nutrition, 64*(6), 850-855.
- Durnin, J. V., & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, 32(1), 77-97.
- Elia, M., Stubbs, R. J., & Henry, C. J. (1999). Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing total starvation. *Obesity Research*, 7(6), 597-604.
- Essen-Gustavsson, B., & Tesch, P. A. (1990). Glycogen and triglyceride utilization in relation to muscle metabolic characteristics in men performing heavy-resistance exercise. *European Journal of Applied Physiology and Occupational Physiology*, 61(1-2), 5-10.

- Fielding, R. A., & Parkington, J. (2002). What are the dietary protein requirements of physically active individuals? New evidence on the effects of exercise on protein utilization during post-exercise recovery.

 Nutrition in Clinical Care, 5(4), 191-196.
- Forbes, G. B. (2000). Body fat content influences the body composition response to nutrition and exercise. *Annals of the New York Academy of Sciences*, *904*(1), 359-365.
- Gallagher, D., Heymsfield, S. B., Heo, M., Jebb, S. A., Murgatroyd, P. R., & Sakamoto, Y. (2000). Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *American Journal of Clinical Nutrition*, 72(3), 694-701.
- Garthe, I., Raastad, T., Refsnes, P. E., Koivisto, A., & Sundgot-Borgen, J. (2011).

 Effect of two different weight-loss rates on body composition and strength and power-related performance in elite athletes.

 International Journal of Sport Nutrition and Exercise Metabolism, 21(2), 97-104.
- Garthe, I., Raastad, T., & Sundgot-Borgen, J. (2011). Long-term effect of weight loss on body composition and performance in elite athletes. International Journal of Sport Nutrition and Exercise Metabolism, 21(5), 426-435.
- Haff, G. G., Koch, A. J., Potteiger, J. A., Kuphal, K. E., Magee, L. M., Green, S. B., & Jakicic, J. J. (2000). Carbohydrate supplementation attenuates muscle glycogen loss during acute bouts of resistance exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 10(3), 326-339.
- Haff, G. G., Stone, M. E., O'Bryant, H. S., Harman, E. A., Dinan, C., Johnson, R.
 L., & Han, K.-H. (1997). Force-Time Dependent Characteristics of
 Dynamic and Isometric Muscle Actions. *Journal of Strength and Conditioning Research*, 11(4), 269-272.
- Hall, K. D. (2007a). Body fat and fat-free mass inter-relationships: Forbes's theory revisited. *British Journal of Nutrition*, *97*(06), 1059-1063.

- Hall, K. D. (2007b). What is the required energy deficit per unit weight loss? *International Journal of Obesity, 32*(3), 573-576.
- Halson, S. L., Bridge, M. W., Meeusen, R., Busschaert, B., Gleeson, M., Jones, D. A., & Jeukendrup, A. E. (2002). Time course of performance changes and fatigue markers during intensified training in trained cyclists. *Journal of Applied Physiology*, *93*(3), 947-956.
- Halton, T. L., & Hu, F. B. (2004). The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *Journal of the American College of Nutrition*, *23*(5), 373-385.
- Hämäläinen, E. K., Adlercreutz, H., Puska, P., & Pietinen, P. (1983). Decrease of serum total and free testosterone during a low-fat high-fibre diet. *Journal of Steroid Biochemistry*, 18(3), 369-370.
- Hämäläinen, E. K., Adlercreutz, H., Puska, P., & Pietinen, P. (1984). Diet and serum sex hormones in healthy men. *Journal of Steroid Biochemistry,* 20(1), 459-464.
- Hopkins, W. G. (2012a). newstats.org/xPostOnlyCrossover.xls.
- Hopkins, W. G. (2012b). newstats.org/xPrePostCrossover.xls.
- Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009).

 Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*, 41(1), 3-13.
- IOC consensus statement on sports nutrition 2010. (2011). *Journal of Sports Sciences, 29 Suppl 1*, S3-4.
- Kim, H., Lee, S., & Choue, R. (2011). Metabolic responses to high protein diet in Korean elite bodybuilders with high-intensity resistance exercise.

 Journal of the International Society of Sports Nutrition, 8, 10.
- Kleiner, S. M., Bazzarre, T. L., & Litchford, M. D. (1990). Metabolic profiles, diet, and health practices of championship male and female bodybuilders. *Journal of the American Dietetic Association*, *90*(7), 962-967.
- Kopple, J. D. (1987). Uses and limitations of the balance technique. *JPEN: Journal of Parenteral and Enteral Nutrition, 11*(5 Suppl), 79S-85S.

- Koral, J., & Dosseville, F. (2009). Combination of gradual and rapid weight loss: effects on physical performance and psychological state of elite judo athletes. *Journal of Sports Sciences*, *27*(2), 115-120.
- Koutedakis, Y., Pacy, P. J., Quevedo, R. M., Millward, D. J., Hesp, R., Boreham, C., & Sharp, N. C. (1994). The effects of two different periods of weight-reduction on selected performance parameters in elite lightweight oarswomen. *International Journal of Sports Medicine*, 15(8), 472-477.
- Lambert, C. P., Frank, L. L., & Evans, W. J. (2004). Macronutrient considerations for the sport of bodybuilding. *Sports Medicine*, *34*(5), 317-327.
- Layman, D. K., & Baum, J. I. (2004). Dietary protein impact on glycemic control during weight loss. *Journal of Nutrition*, *134*(4), 968S-973S.
- Layman, D. K., Boileau, R. A., Erickson, D. J., Painter, J. E., Shiue, H., Sather, C., & Christou, D. D. (2003). A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. *Journal of Nutrition*, 133(2), 411-417.
- Layman, D. K., Evans, E., Baum, J. I., Seyler, J., Erickson, D. J., & Boileau, R. A. (2005). Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *Journal of Nutrition*, 135(8), 1903-1910.
- Layman, D. K., Shiue, H., Sather, C., Erickson, D. J., & Baum, J. (2003).

 Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. *Journal of Nutrition*, 133(2), 405-410.
- Leidy, H. J., Armstrong, C. L., Tang, M., Mattes, R. D., & Campbell, W. W. (2010). The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. *Obesity* (Silver Spring), 18(9), 1725-1732.
- Lemon, P. W. (2000). Beyond the zone: Protein needs of active individuals.

 Journal of the American College of Nutrition, 19(suppl 5), 513S-521S.
- Lemon, P. W., Tarnopolsky, M. A., MacDougall, J. D., & Atkinson, S. A. (1992).

 Protein requirements and muscle mass/strength changes during

- intensive training in novice bodybuilders. *Journal of Applied Physiology*, 73(2), 767-775.
- Leveritt, M., & Abernethy, P. J. (1999). Effects of Carbohydrate Restriction on Strength Performance. *Journal of Strength and Conditioning Research*, 13(1), 52-57.
- Linn, T., Santosa, B., Gronemeyer, D., Aygen, S., Scholz, N., Busch, M., & Bretzel, R. G. (2000). Effect of long-term dietary protein intake on glucose metabolism in humans. *Diabetologia*, *43*(10), 1257-1265.
- Liu, Z., & Barrett, E. J. (2002). Human protein metabolism: its measurement and regulation. *American Journal of Physiology. Endocrinology and Metabolism*, 283(6), E1105-1112.
- Loucks, A. B., Verdun, M., & Heath, E. M. (1998). Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. *Journal of Applied Physiology*, *84*(1), 37-46.
- MacDougall, J. D., Ray, S., Sale, D. G., McCartney, N., Lee, P., & Garner, S.(1999). Muscle substrate utilization and lactate production. *Canadian Journal of Applied Physiology*, 24(3), 209-215.
- Maestu, J., Eliakim, A., Jurimae, J., Valter, I., & Jurimae, T. (2010). Anabolic and catabolic hormones and energy balance of the male bodybuilders during the preparation for the competition. *Journal of Strength and Conditioning Research*, 24(4), 1074-1081.
- Marchini, J. S., Castillo, L., Chapman, T. E., Vogt, J. A., Ajami, A., & Young, V. R. (1993). Phenylalanine conversion to tyrosine: comparative determination with L-[ring-2H5]phenylalanine and L-[1-13C]phenylalanine as tracers in man. *Metabolism: Clinical and Experimental*, 42(10), 1316-1322.
- Mero, A. A., Huovinen, H., Matintupa, O., Hulmi, J. J., Puurtinen, R., Hohtari, H., & Karila, T. (2010). Moderate energy restriction with high protein diet results in healthier outcome in women. *Journal of the International Society of Sports Nutrition*, 7(1), 4.

- Mettler, S., Mitchell, N., & Tipton, K. D. (2010). Increased protein intake reduces lean body mass loss during weight loss in athletes. *Medicine and Science in Sports and Exercise*, 42(2), 326-337.
- Millward, D. J. (2004). Macronutrient intakes as determinants of dietary protein and amino acid adequacy. *Journal of Nutrition*, *134*(6), 1588S-1596S.
- Millward, D. J., & Rivers, J. P. (1988). The nutritional role of indispensable amino acids and the metabolic basis for their requirements. *European Journal of Clinical Nutrition*, *42*(5), 367-393.
- Millward, D. J., & Rivers, J. P. (1989). The need for indispensable amino acids: the concept of the anabolic drive. *Diabetes/Metabolism Reviews*, *5*(2), 191-211.
- Morgan, W. P., Costill, D. L., Flynn, M. G., Raglin, J. S., & O'Connor, P. J. (1988).

 Mood disturbance following increased training in swimmers. *Medicine*and Science in Sports and Exercise, 20(4), 408-414.
- Mourier, A., Bigard, A. X., de Kerviler, E., Roger, B., Legrand, H., & Guezennec,
 C. Y. (1997). Combined effects of caloric restriction and branchedchain amino acid supplementation on body composition and exercise
 performance in elite wrestlers. *International Journal of Sports Medicine*, 18(1), 47-55.
- Nair, K. S., Woolf, P. D., Welle, S. L., & Matthews, D. E. (1987). Leucine, glucose, and energy metabolism after 3 days of fasting in healthy human subjects. *American Journal of Clinical Nutrition*, 46(4), 557-562.
- Newton, L. E., Hunter, G. R., Bammon, M., & Roney, R. K. (1993). Changes in psychological state and self-reported diet during various phases of training in competitive bodybuilders. *Journal of Strength and Conditioning Research*, 7(3), 153-158.
- Nicholls, A. R., Backhouse, S. H., Polman, R. C., & McKenna, J. (2009). Stressors and affective states among professional rugby union players.

 Scandinavian Journal of Medicine and Science in Sports, 19(1), 121-128.

- Oddoye, E. A., & Margen, S. (1979). Nitrogen balance studies in humans: Longterm effect of high nitrogen intake on nitrogen accretion. *Journal of Nutrition*, *109*(3), 363-377.
- Pasiakos, S. M., Cao, J. J., Margolis, L. M., Sauter, E. R., Whigham, L. D., McClung, J. P., . . . Young, A. J. (2013). Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: a randomized controlled trial. *FASEB Journal*, *27*(9), 3837-3847.
- Peterson, M. D., Rhea, M. R., & Alvar, B. A. (2005). Applications of the dose-response for muscular strength development: a review of meta-analytic efficacy and reliability for designing training prescription. *Journal of Strength and Conditioning Research*, 19(4), 950-958.
- Peterson, M. J., Czerwinski, S. A., & Siervogel, R. M. (2003). Development and validation of skinfold-thickness prediction equations with a 4-compartment model. *American Journal of Clinical Nutrition*, 77(5), 1186-1191.
- Phillips, S. M. (2004). Protein requirements and supplementation in strength sports. *Nutrition*, *20*(7-8), 689-695.
- Phillips, S. M. (2006). Dietary protein for athletes: from requirements to metabolic advantage. *Applied Physiology, Nutrition, and Metabolism,* 31(6), 647-654.
- Phillips, S. M., Moore, D. R., & Tang, J. E. (2007). A critical examination of dietary protein requirements, benefits, and excesses in athletes.

 International Journal of Sport Nutrition and Exercise Metabolism, 17

 Suppl, S58-76.
- Phillips, S. M., & Van Loon, L. J. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *Journal of Sports Sciences, 29 Suppl 1*, S29-38.
- Pikosky, M. A., Smith, T. J., Grediagin, A. N. N., Castaneda-Sceppa, C., Byerley, L. O., Glickman, E. L., & Young, A. J. (2008). Increased protein maintains nitrogen balance during exercise-induced energy deficit.

 Medicine and Science in Sports and Exercise, 40(3), 505-512

- Rossow, L. M., Fukuda, D. H., Fahs, C. A., Loenneke, J. P., & Stout, J. R. (2013).

 Natural Bodybuilding Competition Preparation and Recovery: A 12
 Month Case Study. *International journal of sports physiology and performance*, [Epub ahead of print].
- Rushall, B. S. (1990). A tool for measuring stress tolerance in elite athletes. *Journal of Applied Sport Psychology*, 2(1), 51-66.
- Sallinen, J., Pakarinen, A., Ahtiainen, J., Kraemer, W. J., Volek, J. S., & Häkkinen, K. (2004). Relationship Between Diet and Serum Anabolic Hormone Responses to Heavy-Resistance Exercise in Men.

 International Journal of Sports Medicine, 25(8), 627-633.
- Sandoval, W. M., & Heyward, V. H. (1991). Food selection patterns of bodybuilders. *International Journal of Sport Nutrition*, *1*(1), 61-68.
- Sandoval, W. M., Heyward, V. H., & Lyons, T. M. (1989). Comparison of body composition, exercise and nutritional profiles of female and male body builders at competition. *Journal of Sports Medicine and Physical Fitness*, *29*(1), 63-70.
- Saudek, C. D., & Felig, P. (1976). The metabolic events of starvation. *American Journal of Medicine*, 60(1), 117-126.
- Shacham, S. (1983). A Shortened Version of the Profile of Mood States. *Journal of Personality Assessment, 47*(3), 305.
- Short, K. R., Meek, S. E., Moller, N., Ekberg, K., & Nair, K. S. (1999). Whole body protein kinetics using Phe and Tyr tracers: an evaluation of the accuracy of approximated flux values. *American Journal of Physiology,* 276(6 Pt 1), E1194-1200.
- Slater, G., & Phillips, S. M. (2011). Nutrition guidelines for strength sports:
 Sprinting, weightlifting, throwing events, and bodybuilding. *Journal of Sports Sciences*, 29(sup1), S67-77.
- Smeets, A. J., Soenen, S., Luscombe-Marsh, N. D., Ueland, O., & Westerterp-Plantenga, M. S. (2008). Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *Journal of Nutrition*, 138(4), 698-702.

- Smith, G. I., Villareal, D. T., & Mittendorfer, B. (2007). Measurement of human mixed muscle protein fractional synthesis rate depends on the choice of amino acid tracer. *American Journal of Physiology. Endocrinology and Metabolism*, 293(3), E666-671.
- Stiegler, P., & Cunliffe, A. (2006). The role of diet and exercise for the maintenance of fat-free mass and resting metabolic rate during weight loss. *Sports Medicine*, *36*(3), 239-262.
- Stone, M. H., Sanborn, K., O'Bryant, H. S., Hartman, M., Stone, M. E., Proulx, C., . . . Hruby, J. (2003). Maximum strength-power-performance relationships in collegiate throwers. *Journal of Strength and Conditioning Research*, *17*(4), 739-745.
- Suryanarayana, B. V., Kent, J. R., Meister, L., & Parlow, A. F. (1969). Pituitary-gonadal axis during prolonged total starvation in obese men. *American Journal of Clinical Nutrition*, 22(6), 767-770.
- Tarnopolsky, M. A. (2008). Building muscle: nutrition to maximize bulk and strength adaptations to resistance exercise training. *European journal of sport science*, 8(2), 67-76.
- Tarnopolsky, M. A., Atkinson, S. A., MacDougall, J. D., Chesley, A., Phillips, S., & Schwarcz, H. P. (1992). Evaluation of protein requirements for trained strength athletes. *Journal of Applied Physiology*, 73(5), 1986-1995.
- Tarnopolsky, M. A., MacDougall, J. D., & Atkinson, S. A. (1988). Influence of protein intake and training status on nitrogen balance and lean body mass. *Journal of Applied Physiology*, *64*(1), 187-193.
- Tipton, K. D. (2008). Protein for adaptations to exercise training. *European journal of sport science*, 8(2), 107-118.
- Tipton, K. D. (2011). Efficacy and consequences of very-high-protein diets for athletes and exercisers. *Proceedings of the Nutrition Society, 70*(2), 205-214.
- Tipton, K. D., & Wolfe, R. R. (2004). Protein and amino acids for athletes. *Journal of Sports Sciences*, 22(1), 65-79.

- Turocy, P. S., DePalma, B. F., Horswill, C. A., Laquale, K. M., Martin, T. J., Perry, A. C., . . . Utter, A. C. (2011). National Athletic Trainers' Association Position Statement: Safe Weight Loss and Maintenance Practices in Sport and Exercise. *Journal of Athletic Training*, 46(3), 322-336.
- Umeda, T., Nakaji, S., Shimoyama, T., Yamamoto, Y., Totsuka, M., & Sugawara, K. (2004). Adverse effects of energy restriction on myogenic enzymes in judoists. *Journal of Sports Sciences*, *22*(4), 329-338.
- van der Ploeg, G. E., Brooks, A. G., Withers, R. T., Dollman, J., Leaney, F., & Chatterton, B. E. (2001). Body composition changes in female bodybuilders during preparation for competition. *European Journal of Clinical Nutrition*, *55*(4), 268-277.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., . . . Westerterp-Plantenga, M. (2008). Protein-induced satiety: effects and mechanisms of different proteins. *Physiology and Behavior*, *94*(2), 300-307.
- Volek, J. S., Kraemer, W. J., Bush, J. A., Incledon, T., & Boetes, M. (1997).
 Testosterone and cortisol in relationship to dietary nutrients and resistance exercise. *Journal of Applied Physiology*, 82(1), 49-54.
- Wagenmakers, A. J. (1999). Tracers to investigate protein and amino acid metabolism in human subjects. *Proceedings of the Nutrition Society,* 58(4), 987-1000.
- Walberg-Rankin, J., Edmonds, C. E., & Gwazdauskas, F. C. (1993). Diet and weight changes of female bodybuilders before and after competition.

 International Journal of Sport Nutrition, 3(1), 87-102.
- Walberg, J. L., Leidy, M. K., Sturgill, D. J., Hinkle, D. E., Ritchey, S. J., & Sebolt,
 D. R. (1988). Macronutrient content of a hypoenergy diet affects
 nitrogen retention and muscle function in weight lifters. *International Journal of Sports Medicine*, 9(4), 261-266.
- West, D. W., & Phillips, S. M. (2011). Associations of exercise-induced hormone profiles and gains in strength and hypertrophy in a large cohort after weight training. *European Journal of Applied Physiology*, 112(7), 2693-2702.

- Westerterp-Plantenga, M. S. (2008). Protein intake and energy balance.

 *Regulatory Peptides, 149(1-3), 67-69.
- Wilson, J., & Wilson, G. J. (2006). Contemporary issues in protein requirements and consumption for resistance trained athletes. *Journal of the International Society of Sports Nutrition*, *3*(1), 7-27.
- Withers, R. T., Noell, C. J., Whittingham, N. O., Chatterton, B. E., Schultz, C. G.,
 & Keeves, J. P. (1997). Body composition changes in elite male
 bodybuilders during preparation for competition. *Australian Journal of Science and Medicine in Sport, 29*(1), 11-16.
- Wolfe, R. R. (2006). Skeletal muscle protein metabolism and resistance exercise. *Journal of Nutrition*, 136(2), 525S-528S.
- Wolfe, R. R., Wolfe, M. H., Nadel, E. R., & Shaw, J. H. (1984). Isotopic determination of amino acid-urea interactions in exercise in humans. Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 56(1), 221-229.
- Yuhasz, M. S. (1974). *Physical Fitness Manual*. London, Canada: University of Western Ontario.
- Zak, R., Martin, A. F., & Blough, R. (1979). Assessment of protein turnover by use of radioisotopic tracers. *Physiological Reviews*, *59*(2), 407-447.

APPENDICES

Appendix 1: Ethics approval, Auckland University of Technology Ethics Committee



4 December 2012

Caryn Zinn Faculty of Health and Environmental Sciences

Dear Carvn

Re Ethics Application: 12/313 The effects of two protein intakes (1.6g/kg to 2.8g/kg) on fat free mass and performance in lean weight lifters during a hypoenergetic diet.

Thank you for providing evidence as requested, which satisfies the points raised by the AUT University Ethics Committee (AUTEC).

Your ethics application has been approved for three years until 3 December 2015.

Acting under delegated authority and subject to endorsement by AUTEC at its meeting of 4 February 2013, the Executive Secretary approved the satisfactory resolution of AUTEC's condtions.

As part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online throughhttps://www.aut.ac.nz/research/research-ethics/ethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 3 December 2015;
- A brief report on the status of the project using form EA3, which is available online throughhttps://www.aut.ac.nz/research/research-ethics/ethics. This report is to be submitted either when the approval expires on 3 December 2015 or on completion of the project.

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to obtain this. If your research is undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply there.

To enable us to provide you with efficient service, please use the application number and study title in all correspondence with us. If you have any enquiries about this application, or anything else, please do contact us at ethics@aut.ac.nz.

All the very best with your research,

april O

Dr Rosemary Godbold Executive Secretary

Auckland University of Technology Ethics Committee

CC. eric.helms@aut.ac.nz; John Cronin

Appendix 2: Participant information sheet



Participant Information Sheet

Date Information Sheet Produced:

26 September 2012

Project Title

The effects of two macronutrient combinations on fat free mass and performance in lean weight lifters during a hypoenergetic diet

An Invitation

Hello, my name is Eric Helms and I am inviting you to join me in a research project related to nutrient intake during weight training and weight loss for the completion of my Masters. Participation is entirely voluntary, and you may withdraw at any time from the study. If you are a student, athlete, or in any way affiliated with AUT or a partner organization, your choice to participate in this study or not will neither advantage nor disadvantage you.

What is the purpose of this research?

The purpose of this research is to determine which of two macronutrient combinations is more favourable while undergoing a calorically-restricted diet while training. An example of when this would occur would be the case of weight-class athletes dieting to make a cut off for their weight division. This information will assist in establishing optimal nutritional strategies to maintain performance and lean body mass in these athletes under these conditions.

How was I identified and why am I being invited to participate in this research?

You have been identified by personally responding to an email or flyer that advertises this research. If you are receiving this information sheet, you should have already contacted me to express your interest and believe that you meet the inclusion criteria for this study. If you believe you are receiving this information sheet in error, please contact the researcher at erric.helms@aut.ac.nz.

To participate in this study you must be a healthy male between the ages of 20-40, who has been consistently strength training for a full year or more, preferably with barbell weight training experience. We are looking for participants who are not using anabolic steroids or any other illegal performance enhancing drugs, who are lean (low in body fat percentage), and have been weight-stable for a period of one month or longer.

What will happen in this research?

Before participating in this study read over this information sheet fully. Along with this sheet, you should have received registration paperwork and an informed consent form via email. Complete and email the forms back to me including the informed consent paperwork and we will schedule an initial screening session. I will explain the details of the study fully in person and I will obtain your verbal consent before we commence the preliminary testing for final selection and of course you can withdraw at any time.

You will be tested to assess body composition and technique in the barbell back squat and bench press and we will also have a chat about the procedures and details of participation. This will include skin fold calliper tests, and supervised performance of the two lifts with a very light weight. This testing may include instruction on technique as determined on a case by case basis. Based on the results of these tests, the final participants will be selected to participate in the full project.

The full project involves undergoing two periods of caloric restriction with differing macro nutrient combinations. Food plans and supplement powders will be provided during two 14 day periods that you are to follow. The two periods will be one month apart. Before and after each 14 day period, a full day of testing will occur. We will be performing MRI scans, assessing skin fold thickness with callipers, taking measurements of body circumferences and lengths, testing for changes in muscle mass, strength, and athletic performance.

Prior to being provided with food plans and supplement powders for the 14 day periods, you will be taught how to track your nutritional intake, and you will be provided with journals. For a period of one week you will be asked to track your diet, and at the completion of this week you will turn in your journals to the researchers. This will inform us on how to design your diets for the study. After submitting your journals, the researchers, including a registered dietician, will use them to write you a diet with a modified macronutrient intake to follow for the intervention diet to follow. If any journals are incomplete or inaccurate to the point where we cannot properly design a diet for a participant, we will have to exclude that participant from the study.

During the 14 day periods of caloric restriction, participants will be asked to follow their normal training schedule, with only slight modifications (when needed) to include the barbell bench press and back squat. Outside of the supplement powder and the meals on the food plans provided, only calorie free beverages may be consumed. The food plans provided will be designed based on your current diet. However, diets will be modified to create a reduction in calories. To meet the macronutrient combinations that we are studying, you are supplied a powder in addition to your diet plans. You will be instructed to consume the supplement powders mixed with water in 1-3 shakes per day. These powders are one of two combinations of macronutrients (carbohydrate, fat and protein). Both the participants and researchers will not know which powder you are receiving until after the study is over. Every day during the 14 day interventions, participants will complete two self administered questionnaires related to daily stress levels.

For this project, participants will be divided into two groups. Each group will receive a different macronutrient combination; however, the supplements and food plans won't disclose what the specific combinations are to prevent participants from expecting certain results and compensating in either training or eating. Likewise, the primary researcher will also be unaware as to which dietary intervention you are following at any time. Both groups will be switched after the one month period between testing periods, so both groups will experience both interventions at different times. It is important to prevent participants from knowing which intervention they are following to prevent the "placebo effect" and to prevent

the researchers from knowing to eliminate bias. This helps to maintain the validity of the results.

What are the discomforts and risks?

Strength tests, such as the one rep maximum bench press and squat, carry the same inherent risk that weight training carries. There is the potential of injury, although the risk is minimal. Testing of muscular endurance, anaerobic power, and muscular power carry negligible risks, but create fatigue and discomfort similar to intense exercise.

The anticipated stresses to be encountered during the interventions of this study will not be outside of those risks performed during your regular training and nutritional regimes. You may experience hunger over the period of caloric restriction but it is not anticipated to be significant. The caloric reduction is within standard practices for healthy dieting and weight loss and the caloric restriction is short term, lasting only 14 days. Furthermore, while we have tried to make the supplement powders as palatable as possible, some may find they do not enjoy or even dislike the taste of the supplement powder.

While the actual intervention should be negligible in terms of discomfort and embarrassment, the collection of body measurements and the analysis of body composition may potentially create embarrassment or discomfort.

MRI scanning requires you to hold still for an extended period of time (approximately one hour total in this case) in a confined space and is loud, and skin calliper testing requires skin folds to be pinched by callipers while in undergarments.

Lastly, the daily stress questionnaires you will fill out during the intervention may contain questions which could provide embarrassment.

How will these discomforts and risks be alleviated?

The risk of strength and performance testing is likely reduced compared to your habitual training. You will have completed a PAR-Q health questionnaire in order to be included, the primary researcher is first aid, CPR and AED certified, and spotters will be in use during testing. The researchers are qualified professionals in the field of sports performance testing and are aware of and will be implementing all relevant safety procedures to ensure the minimization of risk.

The MRI testing may be quite noisy so we recommend you bring headphones and music, or you have the option of using ear plugs which we will provide. We will perform body composition analysis in private, with only a researcher, and the specialist performing the tests present in the room with you. Verbal check-ins will be made to ensure you understand and are still comfortable with going forward prior to any testing. You can decline continuation in the study at any time without reprisal

Lastly, the stress questionnaires will be taken in private, and will only be identified by an encoded number, not by personally identifiable information.

What are the benefits?

During preliminary screening for selection, you will receive professional instruction on the barbell squat and bench press if you are not proficient in the lifts already. Also, you will receive body composition testing by an accredited anthropometrist. If included in the full study, you will learn how to track your nutrition, and you will be provided with food journals for a total of 28 days of food tracking. Lastly, you

will receive a battery of body composition and performance assessments a total of four times. This valuable information can be used to guide your future training and nutritional plans in order to further your fitness or athletic pursuits.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

How will my privacy be protected?

We will fully disclose the details of the study beforehand; as to who you will be interacting with, who will perform your tests and assessments, who will analyze the data, what it will be used for, and we will go over this verbally, and also provide an informed consent form in writing.

Your personal information will be removed from the data collection and the study. We will assign each participant a numerical identifier, so that your personal information is not present in the study or during the data collection or analysis. Furthermore, your contact details will be kept separately from this information.

Your information will be stored for future use in research. However, your contact details will not be attached to the study results. Furthermore, this information will only be used in the context of research that is performed by AUT researchers or by affiliate researchers. And it will be stored with personal details removed. You will not be contacted by third parties and your information will be protected for any use outside of those outlined.

What are the costs of participating in this research?

All that we require is your time and effort which we are sincerely grateful for. After completing the paperwork (registration, health questionnaire, and informed consent) prior to participating in the full study, the initial calliper and exercise form tests will take between thirty minutes to an hour for a potential period of two full hours per participant.

For participants included in the full study, they will be given detailed instructions in writing on how to track their habitual diet. This will occur a few days after technique and body composition analysis and will take two to three hours in total to read, understand and clarify depending on what questions you have about diet tracking.

The first week of dietary tracking will require you to write down all food and beverages consumed; this may take five to ten minutes per meal consumed.

After this tracking period, you will be supplied supplement powder and a food plan for 14 days and the time and effort required will be reduced as you simply need to consume the food on the plan and powder provided by the end of the day.

Before starting the dietary intervention, pre testing will occur, which includes performance tests, body composition analyses, and MRI scans. Thus, the majority of a day will be required for testing before the start of the intervention diets. By far, these pre and post testing sessions will carry the greatest burden on your time.

The only additional time consuming activity in the 14 day intervention periods will be the daily completion of the stress questionnaires, which will take at most half an hour per day.

At the end of the 14 day intervention, a full day will be required for post testing much like the pre testing. Then you will be released for a one month period. In the last two weeks of this month, you will receive an email from the researcher instructing you to return to your habitual dietary practices if you are not already doing so (we will provide you the data from the first week of dietary tracking to help). After this month, we will repeat the process, supplying you with a new food plan and supplement powder, undergoing a pre testing session, and then a post testing session after 14 days on the diet. In total, you will do four one-day testing sessions. These sessions will require the most time, keeping you busy most of the day.

Once analysis is complete, individual results and the study results will be disseminated to you via email and we will be available for contact regarding the findings.

What opportunity do I have to consider this invitation?

We are planning to run participants through the intervention starting in January and February to give enough time to analyze the data. From starting the initial screening, the entire study will take 3 months including the 1 month break between interventions. So, please make sure to decide on whether you would like to participate by early to mid January at the latest.

How do I agree to participate in this research?

Please complete the health questionnaire, the consent form and the registration paperwork that you have been emailed. You can either scan and email this back to me at eric.helms@aut.ac.nz or bring it with you when we schedule your initial screening (it must all be complete before commencing testing though).

Will I receive feedback on the results of this research?

After completion of data collection your personal results will be given to you along with full disclosure of the diet details you followed during each 14 day period. Furthermore, once all the data has been analysed you will receive the results of the study, with the personal information of all participants protected.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, *Dr Caryn Zinn, czinn* @aut.ac.nz, +64 (09) 9219999, ext 7842

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Dr Rosemary Godbold, *rosemary.godbold@aut.ac.nz*, 921 9999 ext 6902.

Whom do I contact for further information about this research?

Researcher Contact Details:

Eric Helms, eric.helms@aut.ac.nz, (09) 921 9999 ext. 5195.

Project Supervisor Contact Details:

Dr Caryn Zinn, czinn@aut.ac.nz, +64 (09) 9219999, ext 7842

Approved by the Auckland University of Technology Ethics Committee on December 4th, AUTEC Reference number **12/313**.

Appendix 3: Participant consent form

Consent Form



Project title: The effects of two macronutrient combinations on fat free
mass and performance in lean weight lifters during a
hypoenergetic diet

Project	Supervisor: Dr Caryn Zinn Primary Researcher: Eric Helms
Please	tick each circle beside each statement if you understand and agree
0	have read and understood the information provided about this
	research project in the Information Sheet dated (dd/mm/yyyy)
0	have had an opportunity to ask questions and to have them answered.
	understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
	am not suffering from heart disease, high blood pressure, any respiratory condition (mild asthma excluded), any illness or injury that mpairs my physical performance, or any infection and I have never been told by a physician that I should not participate in exercise of a nigh intensity
0	have disclosed any injury or illness that will affect my ability to complete the testing
0	acknowledge that there are risks and dangers inherent in physical exercise and declare that I know of no reason why I should not

complete the tests described.

- O I agree to my contact details being stored indefinitely in the SPRINZ research data base in case I need to be contacted for possible follow-up research. An example would be in the case of long-term follow-up studies (not required for participation in this research project).
- O I agree that my de-identified data may be stored indefinitely in the SPRINZ research database, used for future research projects, and shared with other SPRINZ approved researchers without me providing any additional consent beyond this consent. The kind of projects the data might be used for include SPRINZ approved student research for degree completions and international collaborative research (not required for participation in this research project).
- O I agree to take part in this research.
- O I wish to receive a copy of the report from the research (please tick one): YesO NoO

Participant signature:	Date:
Participant name:	
Phone :	Email :
Address :	
	ne #2
Approved by the Auckland University of Technology Et	hics Committee on December 4 th , AUTEC Reference number
12/313	

Note: The Participant should retain a copy of this form.

Appendix 4: Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

Consider the following questions in terms of "YES or NO?"

or NO?"				
1.	YES or NO? (circle one) Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?	1.	Y	N
2.	Do you feel pain in your chest when you do physical activity?	2.	Y	N
3.	In the past month, have you had chest pain when you were not doing physical activity?	3.	Y	N
4.	Do you lose your balance because of dizziness or do you ever lose consciousness?	4.	Y	N
5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?	5.	Y	N
6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?	6.	Y	N
7.	Do you know of any other reason why you should not do physical activity?	7.	Y	N

If you answered YES to one or more questions:

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want as long as you start slowly and build up gradually. Or, you
 may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds
 of activities you wish to participate in and follow his/her advice.
- · Find out which community programs are safe and helpful for you.

If you answered NO to one or more questions:

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active begin slowly and build up gradually. This is the safest and
 easiest way to go.
- Take part in a fitness appraisal this is an excellent way to determine your basic fitness so that you can
 plan the best way for you to live actively.

Delay becoming much more active:

- If you are not feeling well because of a temporary illness such as a cold or a fever wait until you feel better: or
- If you are or may be pregnant talk to your doctor before you start becoming more active.

Informed use of the PAR-Q: Reprinted from ACSM's Health/Fitness Facility Standards and Guidelines, 1997 by American College of Sports Medicine

Appendix 5: Meal plan questionnaire

Meal Plan Questionnaire

(Delete options until your choice remains or write in as directed)

1	.	wou	d	pre	er	to eat	mea	ls	per	day:	
---	---	-----	---	-----	----	--------	-----	----	-----	------	--

- a. 2
- b. 3
- c. 4
- d. 5
- e. 6

2. Breakfast options:

- a. I prefer to eat traditional breakfast food at only breakfast
- b. I don't mind eating traditional breakfast food any time of the day
- Food Aversions, list any foods you are allergic to, are negatively affected by or specifically do not want on your meal plan:
- Food Preferences (delete options you don't want in your meal plan):
 - a. Beef
 - b. Pork
 - c. Lamb
 - d. Veal
 - e. Chicken
 - f. Turkey
 - g. Salmon
 - h. Tuna
 - i. White fish
 - j. Sardines
 - k. Shellfish
 - I. Milk
 - m. Eggs
 - n. Cheese
 - o. Yogurt
 - p. Cottage cheese
 - q. Tofu
 - r. Soy milk
 - s. Breakfast cereals
 - t. Pastas
 - u. Breads
 - v. Rice
 - w. Oatmeal
 - x. Beans
 - v. Lentils
 - z. Peas
 - aa. Apple
 - bb. Banana

- cc. Grapes
- dd. Orange
- ee. Strawberries
- ff. Raspberries
- gg. Blueberries
- hh. Potatoes & yams
- ii. Corn
- jj. Artichoke
- kk. Asparagus
- II. Beets
- mm. Broccoli
- nn. Carrots
- oo. Sprouts
- pp. Celery qq. Peppers
- rr. Tomato
- ss. Avocado
- tt. Peanuts
- uu. Almonds vv. Walnuts
- _
- ww. Pecans

Appendix 6: Profile of Mood States short form

Name:	Date:

POMS SF PROFILE OF MOOD STATES-SHORT FORM

Below is a list of words that describe feelings people have. Please read each one carefully. Then circle ONE answer to the right, which best describes HOW YOU HAVE BEEN FEELING DURING THE **PAST 24 HOURS.**

The numbers refer to these phrases:

0=not at all 1=a little 2=moderately 3=quite a bit 4=extremely

1. Tense 1 2 3 4	20. Discouraged 1 2 3 4
2. Angry 1 2 3 4	21. Resentful 0 1 2 3 4
3. Worn out0 1 2 3 4	22. Nervous0 1 2 3 4
4. Unhappy0 1 2 3 4	23. Miserable 0 1 2 3 4
5. Lively 1 2 3 4	24. Cheerful 0 1 2 3 4
6. Confused0 1 2 3 4	25. Bitter 0 1 2 3 4
7. Peeved 1 2 3 4	26. Exhausted 0 1 2 3 4
8. Sad0 1 2 3 4	27. Anxious 1 2 3 4
9. Active 1 2 3 4	28. Helpless 1 2 3 4
10.On Edge 0 1 2 3 4	29. Weary 1 2 3 4
11.Grouchy 1 2 3 4	30. Bewildered 1 2 3 4
12. Blue0 1 2 3 4	31. Furious0 1 2 3 4
13. Energetic 0 1 2 3 4	32. Full of pep 0 1 2 3 4
14. Hopeless 1 2 3 4	33. Worthless 1 2 3 4
15. Uneasy0 1 2 3 4	34. Forgetful 0 1 2 3 4
16. Restless 1 2 3 4	35. Vigorous 0 1 2 3 4
17. Unable to	36. Uncertain about
Concentrate0 1 2 3 4	things 1 2 3 4
18.Fatigued 1 2 3 4	37. Bushed 0 1 2 3 4
19. Annoyed0 1 2 3 4	

Appendix 7: Daily Analysis of Life Demands of Athletes

Name_					Date
			-		of Life Demands for Athletes (DALDA) Questionnaire
	Part A	A: R	eco	rd s	stress in your life circle a, b, or c (a = worse than normal, b = normal, c = better than normal)
	1.	a	b	c	Diet
	2.	a	b	c	Home Life
	3.	a	b	c	School/college/work
	4.	a	b	c	Friends
	5.				Sports Training
	6.				Climate
					Sleep
	8.				Recreation
	9.				Health
	Total	l "a"	res	pon	ses: Total "b" responses: Total "c" responses:
	Part l	B list	t po	ssil	ble causes of stress circle a, b, or c (a = worse than normal, b = normal, c = better than normal)
	1.				Muscle Pains
	2.	a	b	c	Techniques
	3.	a	b	c	Tiredness
					Need for rest
	5.				Supplementary Work
	6.				Boredom
					Recovery Time
					Irritability
	9.				Weight
	10.	a	b	c	Throat
					Internal
	12.	a	b	c	Unexplained aches
	13.	a	b	c	Technique Strength
					Enough Sleep
	15.				Between Session Recovery
	16.				General Weakness
					Interest
					Arguments
	19.				Skin Rashes
					Congestion
					Training Effort
					Temper
					Swelling
					Likability
	25.				Runny Nose
	Total	"9"	TAC	non	sees: Total "h" reconnees: Total "c" reconnees:

Appendix 8: Baseline dietary tracking information sheet

Baseline Dietary Tracking Information

- Track your diet for 1 week using www.myfitnesspal.com
- At completion send me the login and password details (or make the log public and send me the link to view)
- Use a digital food scale to weigh portions whenever possible.
- Digital food scales are great tools for tracking your nutrition for the future (not only during the study to come). A worthy investment if you would like, these can be purchased relatively cheaply at the warehouse or similar stores \$20-30. (if this is a financial hardship I can provide a loaner scale)
- When eating out, search to find best equivalent of what you ate on www.myfitnesspal.com
- Choose higher calorie options when searching for how to track the food you ate when multiple options exist. Email me if you have questions about the calorie count of a meal eaten out.
- Weigh foods raw before cooking; meat, rice, oats, etc.
- Eat as you do normally!
- www.myfitnesspal.com can be downloaded as an application on smart phones. It can scan barcodes directly into your log and it is a great way to make sure you can still log while away from a computer.
- If you don't have a smart phone, or are more comfortable with a less technology heavy method of tracking, bring a pocket sized notebook and pen with you, and write down serving size and foods you eat and log them when you return home.
- Everything that you eat or drink should be logged; only exceptions are items without calories, i.e. water, non-nutritive artificial sweeteners, and other food labels that show zero calories. Don't forget things like sugar and milk in your tea and blended coffee and condiments all have calories.
- Email me at eric.helms@aut.ac.nz anytime you have an issue with tracking or a question with tracking.

Appendix 9: Study registration form

PARTICIPANT REGISTRATION FORM

Personal Information
Name:
Age:
Email:
Phone:
Study Questionnaire 1) Please be aware of the following inclusion criteria for participation in this study: • Male • 20-40 • At least 1 year of resistance training experience (consistent training for one year with at least 2 sessions per week of vigorous resistance training) • Weight stable for at least one month (weight when measured under similar conditions remains within a 2% range, for example 100kg norm ranging from 98-102kg) • Not using anabolic steroids or other illegal performance enhancing drugs • Willingness to abstain from alcohol consumption during the intervention period Considering these prerequisites, do you believe you meet the inclusion criteria for this study? (If yes, please complete sections below)
Please list your weekly training schedule below as outlined in the below example: Monday
Resistance Training: Barbell Squat 3 sets of 8-12, Leg Curl 3 sets of 8-12, Calf Raise 3 sets of 8-12
Cardiovascular Training: 30 min stationary bike cycling
Monday Resistance Training:
Cardiovascular Training:
Tuesday Resistance Training:
Cardiovascular Training:

Wednesday
Resistance Training:
Cardiovascular Training:
Thursday
Resistance Training:
Cardiovascular Training:
Friday
Resistance Training:
Cardiovascular Training:
Saturday
Resistance Training:
Cardiovascular Training:
Cardovascular Halling.
Sunday
Resistance Training:
Cardiovascular Training:
3) Please circle the level of compliance and accuracy from 1-10 (1 being non compliant and

inaccurate, 10 being perfectly compliant and accurate) that you believe you will have while following a calorically restricted diet plan for two 14 day periods one month apart:

1 2 3 4 5 6 7 8 9 10

Appendix 10: Study recruitment flyer



Do these statements describe you?

I am 20-40 years old

I am male

I have been relatively weight stable for 1 month

I am relatively low in body fat

I have been weight training consistently for at least one year

I don't use anabolic steroids or any other illegal performance enhancing drugs.

I am apparently healthy and not currently diagnosed with any serious medical condition

I would like to participate in a study on dieting and exercise related to weight training and

different combinations of nutrients

If so, you may be qualified to participate in exciting new research examining the effects of a nutritional intervention on lean body mass, strength and performance during weight loss in strength athletes.

If you are interested and willing to contribute your time and energy, please contact the primary researcher Eric Helms by email at: eric.helms@aut.ac.nz





Appendix 11: Sample Participant Meal Plan

Name: Anonymous Diet 1 Plan 1

Meal	Recipes	Directions
Meal 1, 1 piece Egg in a nest Fruit: 160 grams/1 Cup Melon diced, or small apple, or medium orange	Egg in a nest (scaled to 1 piece) 1 extra large Egg 1 slice White, Wheat or Multi-grain bread dash Salt & pepper 7 grams Butter	Cut or tear a 2-inch hole out of the centre of each slice of bread. Melt the butter in a large non-stick frying pan over medium heat until foaming. Add the bread slices. Crack an egg into each bread hole, season with salt and pepper, and cook until the bottoms are golden brown n, about 3 to 4 minutes. Using a flat spatula, flip and cook until the second side is golden brown, about 3 minutes more for runny yolks. Serve immediately.
Meal 2 1 large Kumara (160g) 250 grams Nonfat yogurt	*may use calorie free sweetener (s	plenda stevia equal)
Meal 3 3 serving Easy Grilled Chicken 180 grams Black, pinto, white, kidney or lima beans	Easy Grilled Chicken (scaled to 3 servings) 360q breast, bone and skin removed Chicken breast 175 g/3/4 cup Italian dressing 3/4 Green capsicum (120g) 3/4 Red Capsicum (120g) 3/4 large Zucchini (240g)	Place washed chicken breasts in large sealable bag. Add fat-free Italian dressing and close. Let marinate for 5 to 10 minutes. Cut up capsicum into big chunks, and zucchini into big slices. Put into another sealable bag. Coat with leftover dressing. Grill chicken and veggies over medium heat. Cook canned beans eat with

Notes:

Consume powder in 2-3 shakes per day, just water mixed with the powder, great between meals when hungry.

meal.

May eat in any order or mix ingredients from one meal with another, so long as all food is consumed over 24 hour period.

May add up to 1 cup of green veggies to your day (cucumber, broccoli, snap peas, green beans, spinach, lettuce, zucchini, sprouts or celery)

May swap individual food items for other food items with same carbs, fat, protein and calories.