

Knowledge and perceptions of North-West University rugby players on timing of protein ingestion

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“There is no secret to success. It’s the result of preparation, hard work, and learning from failure.” Colin Powell

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ABSTRACT

Introduction

In South Africa rugby is regarded as a national sport that is being developed from a young age. In universities, rugby players are further being moulded, developed and prepared for the professional league and they often experience pressure to compete at top level (Hale, 2013:3). Training and competition in addition to academic commitments provide unique challenges to university rugby players (Simiyu, 2010:17), not only in terms of optimal time management, but also in terms of optimal nutrition.

The importance of protein for athletes has long been recognized (Tipton & Wolfe, 2004:65) and more recently the correct timing of protein intake has been identified as an important component to optimize the adaptive response to both resistance and endurance exercise. Research conducted by Cermak *et al.* (2014:1454) and McLain *et al.* (2013:68) indicated that timely consumption of protein before, during and after exercise also has the ability to increase muscle protein synthesis, muscle glycogen restoration, muscle damage repair, muscle size, muscle strength and potentially performance. The International Olympic Committee recommends that 20-25g of high quality protein should be ingested 30 minutes following resistance exercise to restore muscle glycogen and promote protein synthesis (Slater & Phillips, 2011:71). Not all rugby players are necessarily familiar with these guidelines and since nutrition knowledge (and perceptions) has been shown to influence what and when athletes eat, (Walsh *et al.*, 2013:371; Strachan *et al.*, 2009:51) information on the knowledge and perceptions of timing of protein intake of university rugby players can be useful to improve nutritional practices via for instance education. Although data is available on nutritional knowledge of rugby players (Alaunyte *et al.*, 2015, Hale, 2013, Walsh *et al.*, 2011) very little is known about university rugby players' knowledge and perceptions specifically regarding the timing of protein intake. Accurate knowledge assessment in a specific population requires a valid and reliable tool to collect knowledge data (Whati *et al.*, 2005:77). To our knowledge, a valid and reliable questionnaire to specifically determine the knowledge of protein timing in university athletes is also not available from the literature. The aim of the dissertation was to therefore determine the knowledge and perceptions of North-West University rugby players on timing of protein intake through a questionnaire that was developed, validated and tested for reliability.

Methods

A descriptive, cross-sectional study with a quantitative and qualitative component was conducted. The study consisted of two main parts including the development and validation of a knowledge questionnaire and a cross-sectional study to determine knowledge and perceptions of university athletes on timing of protein intake. The knowledge questionnaire was developed

by means of a literature review and tested for content validity by experts in the field of sports nutrition. Face validity was determined in a group of students and lectures before the knowledge questionnaire was tested for construct validity (including item difficulty internal consistency and item discrimination) and reliability. The test–retest method was used to test the same questionnaire in 70 hockey and cricket players from North-West University on two separate occasions 15 days apart. Various statistical tests were performed to assess the different aspects of validity including item difficulty index and Cronbach's alpha, and reliability including Cronbach's alpha, t test, percentage difference, correlation coefficients, Kappa statistics and Bland-Altman analysis.

In the second part of the study 103 male rugby players from the North-West University in South-Africa volunteered to complete the knowledge questionnaire on the timing of protein intake. Participants were included if they were between the ages of 18 - 24 years, enrolled in a degree or diploma at the university, and playing for the universities 1st, u/21A or u/19A team or student provincial 1st, u/21A or u/19A team (e.g. these were students from the university who represented the student provincial team or played for the student provincial team). A sub-sample of players per team was also randomly selected to participate in semi-structured focus-group discussions to gain more insight into the perceptions of university rugby players regarding timing of protein intake.

Main Findings

A 12-item knowledge questionnaire was developed. Item difficulty of 11 questions was good (>10%), strength of consistency was good (Cronbach's alpha (CA) = 0.31), but internal consistency was poor (CA = 0.31). The questionnaire is reliable at group level for agreement (t test $P = 0.078$) and association (Bland-Altman indicated 95.7% within limits of agreement). On individual level the questionnaire showed limited bias ($P = 0.072$), limited error (CA = 0.64), limited agreement (Kappa = 0.13), but strong association (Interclass correlation was 0.64). After this newly developed and validated knowledge questionnaire was completed by the rugby players, the overall mean percentage knowledge score on timing of protein intake was $39.8 \pm 13.9\%$ and only 29% of the players scored $\geq 50\%$. Although the majority of participants (87.5%) correctly identified the optimal time to consume protein after training, and 81.6% knew which athletes will benefit from applying protein timing strategies correctly, knowledge regarding the role and benefits, as well as the optimal source and amount of protein to consume when was poor (ranged from 11.7 - 35.5%). The first and u/21 teams tended to score higher on the knowledge questionnaire compared to the u/19 teams (42.0 [33.0 - 50.0] vs. 42.0 [33.0 - 42.0] vs. 33.0 [25.0 - 42.0], $P = 0.06$), however knowledge was not significant different between the forwards and the backs. A spearman rank correlation demonstrated an overall weak positive correlation ($r = 0.24$, $P < 0.05$) between weight and knowledge score. The participants in the

present study perceived the timing of protein intake to be important, but perhaps not as important as the source of protein. The most common perception specifically with regards to timing of protein intake was that the best time to consume protein is after training. The 30-minute period following exercise was perceived as an important period to replenish protein and avoid muscle breakdown. Inter- and intra-reliability of coded focus group discussion data were determined as $\kappa = 0.74$ and $\kappa = 0.81$, respectively.

Conclusion

Although North-West University rugby players perceived protein timing to be important, they have poor knowledge regarding protein timing specifically with regards to the type and amount of protein to be consumed. The fact that they knew when to ingest protein (i.e. after exercise/training) and the perception that protein ingestion is important after exercise could be due to the influence of the coaches instructing them to consume protein after exercise and making protein shakes available to them after exercise. These players should therefore be educated on the timing of protein intake.

Key words

Timing of protein intake, knowledge and perceptions, questionnaire development and validation, university rugby players.

OPSOMMING

Inleiding

In Suid-Afrika word rugby as die nasionale sport beskou en word dit reeds van 'n jong ouderdom af ontwikkel. Op universiteit word rugbyspelers gevorm, ontwikkel en verder voorberei vir die professionele liga en ervaar hulle uiterste druk om op topvlak deel te neem (Hale, 2013:3). Oefening en kompetisie gaan gepaard met akademiese verantwoordelikhede en is intens op universiteit vlak (Simiyu, 2010:17), nie net ten opsigte van optimale tydspannering nie, maar ook ten opsigte van optimale voeding.

Die belangrikheid van proteïene vir atlete word al 'n geruime tyd erken (Tipton & Wolfe, 2004:65) en onlangs het die korrekte tydperk van proteïen-inname gewildheid verwerf as belangrike komponent om aanpassing aan beide weerstands- en uithouvermoë oefeninge te optimaliseer. Navorsing wat deur Cermak *et al.* (2014:1454) en McLain *et al.* (2013:68) gedoen is, het onder andere gewys dat proteïen-inname op die regte tydperk voor, tydens of na oefening, spierproteïen sintese, spierglikogeen herstel, spierherstel, spiergrootte en spierkrag, en potensieel prestasie kan verbeter. Die Internasionale Olimpiese Komitee stel voor dat 20-25g hoë kwaliteit proteïen ingeneem moet word 30-minute na oefening om spierglikogeen te herstel en spierproteïen sintese te bevorder (Slater & Phillips, 2011:71). Nie alle rugbyspelers dra egter kennis van hierdie riglyne nie, en siende dat kennis (en persepsies) 'n invloed het op wat en wanneer atlete eet (Walsh *et al.*, 2013:371; Strachan *et al.*, 2009:51) sal inligting oor universiteit rugbyspelers se kennis oor die tydperk van proteïen-inname baie waardevol wees om voedingspraktyke deur middel van onderrig te verbeter. Alhoewel daar data beskikbaar is rondom die algemene voedingskennis van rugbyspelers (Alaunyte *et al.*, 2015, Hale, 2013, Walsh *et al.*, 2011) is daar nie baie literatuur beskikbaar rondom universiteit rugbyspelers se kennis en persepsies spesifiek oor die tydperk van proteïen-inname nie.

'n Geldige en betroubare vraelys word benodig om akkuraat die kennis van Suid-Afrikaanse universiteit rugbyspelers op die tyd van proteïen-inname te bepaal (Whati *et al.*, 2005:77). Volgens ons kennis is geen geldige en betroubare vraelys in die literatuur beskikbaar om die kennis van universiteits-atlete oor die tydperk van proteïen-inname te toets nie. Die doel van hierdie skripsie was dus om die kennis en persepsies van Noordwes Universiteit (NWU) rugbyspelers op die tydperk van proteïen-inname te bepaal.

Metodes

Hierdie studie het gebruik gemaak van 'n dwarsdeursnee, beskrywende navorsingsontwerp en het kwantitatiewe en kwalitatiewe komponente ingesluit. Die studie het uit twee hoofdele bestaan naamlik die ontwerp en validering van 'n kennisvraelys, asook 'n dwarsdeursnee studie

om die kennis en persepsies van universiteits-atlete rondom die tydstip van proteïen-inname te bepaal. Die vraelys is ontwikkel deur 'n deeglike literatuur oorsig om relevante konsepte te identifiseer en is getoets vir geldigheid van die inhoud (inhoudsgeldigheid) deur kenners in die veld van sportvoeding. Geldigheid en duidelikheid van die vraelys is getoets deur dosente en studente van die NWU voordat die vraelys getoets is vir geldigheid van hoe vroeë op die algehele vraelys by mekaar pas (insluitend item moeilikheidsgraad indeks, interne konsekwenheid en item diskriminasie) en betroubaarheid. Die toets-hertoets metode is gebruik om dieselfde kennisvraelys in 70 hokkie en krieketspelers van NWU op twee geleenthede 15-dae uit mekaar te toets. Verskeie statistiese toetse is uitgevoer om verskillende aspekte van geldigheid insluitende "item difficulty index" en Cronbach's alfa, en betroubaarheid insluitende Cronbach's alfa, t toets, persentasie verskil, korrelasie koëffisiënt, Kappa statistieke en Bland-Altman analise te ondersoek. Nadat daar vasgestel is dat die vraelys geldig en betroubaar is het 103 NWU rugbyspelers die vraelys voltooi om hulle kennis rondom die tyd van proteïen-inname te bepaal. Deelnemers is ingesluit as hulle tussen 18 - 24 jaar oud was, geregistreer was vir 'n kursus aan die NWU en vir die 1ste, o/21A of o/19A spanne van die NWU of provinsiale studente spanne gespeel het. Semi-gestruktureerde fokus groep besprekings is uitgevoer om persepsie rondom tyd van proteïen-inname vas te stel.

Resultate

'n Vraelys met 12 vrae is ontwikkel. Die moeilikheidsgraad volgens die "item difficulty index" van 11 vrae was goed (>10%), sterkte en konsekwenheid was aanvaarbaar (CA = 0.31), maar interne konsekwenheid was swak (CA = 0.31). Die vraelys is betroubaar op groep vlak vir ooreenkoms (t toets $P = 0.078$) en assosiasie (Bland-Altman dui 95.7% binne die grense van ooreenkoms aan). Op individuele vlak dui die vraelys beperkte vooroordeel ($P = 0.072$), foute (CA = 0.64) en ooreenkoms (Kappa = 0.13) aan, maar sterk assosiasie (Interklas korrelasie = 0.64). Nadat die nuut ontwikkelde vraelys deur die NWU rugby spelers voltooi is, was die gemiddelde persentasie vir hulle kennis oor die tydstip van proteïen-inname $39.8 \pm 13.9\%$ en net 29% het $\geq 50\%$ behaal. Alhoewel die meerderheid van die spelers (87.5%) kon aandui wanneer die beste tyd is om proteïen na oefening in te neem, en 81.6% geweet het watter atlete sal baat vind by strategieë wat fokus op die regte tydstip van proteïen-inname, was hulle kennis rondom die funksie en voordele, sowel as die optimale bron en hoeveelheid proteïen om in te neem, swak (11.7 - 35.5%). Die 1ste en o/21 spanne het geneig om beter te presteer op die kennisvraelys in vergelyking met die o/19 spanne ($42.0 [33.0 - 50.0]$ vs. $42.0 [33.0 - 42.0]$ vs. $33.0 [25.0 - 42.0]$, $P = 0.06$), maar daar was geen beduidende verskil tussen die uitslae van die voorspelers en agterspelers nie. Spearman rang korrelasie demonstreer 'n algehele swak positiewe korrelasie ($r = 0.236$, $P = 0.016$) tussen gewig en vlak van kennis. Die deelnemers in hierdie studie het die tydstip van proteïen-inname as belangrik geag, maar dalk nie so belangrik soos die bron van proteïen wat ingeneem moet word nie. Die mees algemene persepsie

rondom spesifiek die tydstip van proteïen-inname, was dat die beste tyd om proteïene in te neem, na oefening is. Hulle het verder bevestig dat 30 minute na oefening 'n belangrike tyd is om proteïen in te neem om sodoende spier afbraak te verhoed. Inter- en intra- betroubaarheid van die fokus groep besprekings was goed ($\kappa = 0.74$ en $\kappa = 0.8$, onderskeidelik).

Gevolgtrekking

Alhoewel die rugbyspelers die tydstip van proteïen-inname as belangrik geag het, was hulle kennis oor die tydstip van proteïen-inname swak, spesifiek met betrekking tot die funksie en voordele, sowel as die optimale bron en hoeveelheid proteïen om in te neem. Hierdie spelers kan dus baat vind by onderrig oor die tydstip van proteïen-inname.

Sleutel terme

Tydstip van proteïen-inname; kennis en persepsies; vraelys ontwikkeling en validering, universiteit rugbyspelers.

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LIST OF ABBREVIATIONS

AAs	Amino Acids
ACSM	American College of Sports Medicine
ADA	American Dietetic Association
BCAA	Branched Chain Amino Acid
BF	Body Fat
BM	Body Mass
BW	Body Weight
CHO	Carbohydrate
CON	Control Group
CK	Creatine Kinase
DRI	Daily Recommended Intake
EAA	Essential Amino Acid
EXP	Experimental Group
FFM	Fat Free Mass
FM	Fat Mass
g	Gram
GH	Growth Hormone
HCL	Hydrochloric Acid
INT	Intermediate
IOC	International Olympic Committee
ISSN	International Society for Sports Nutrition
Kcal	Kilocalorie

kg	Kilogram
kJ	Kilojoule
LBM	Lean Body Mass
Mod	Moderate
MPS	Muscle Protein Synthesis
MPB	Muscle Protein Breakdown
NBAL	Net Balance
NEAA	Non-essential amino acid
NH ₂	Ammonia
NPB	Net Protein Balance
NT	Nutrient Timing
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PLC	Placebo
Prot	Protein
RE	Recommended Dietary Allowance
RM	Repetition Maximum
RTF	Run Time to Fatigue
SD	Standard Deviation
TC	Total Cholesterol
TEE	Total Energy Expenditure
WHO	World Health Organization

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CHAPTER 1: INTRODUCTION

1.1 Background

Over the past 20 years, researchers have documented how optimal nutrition benefits exercise performance (Kreider *et al.*, 2010:1). Although good nutritional choices do not compensate for talent or lack of training, optimal nutrition will help talented and motivated athletes to make the most of their potential (Maughan, 2007:103).

Protein in particular is considered an important nutritional factor and “building block” not only for athletes competing in strength and power sports, but also for endurance athletes (Tipton *et al.*, 2007:17; Phillips & van Loon, 2011:29). Protein plays an important role in maximising muscle protein synthesis, increasing glycogen resynthesis, increasing muscle strength, repairing muscle damage, promoting training adaptations and enhancing body composition (Cermak *et al.*, 2012:1454). These benefits may translate into improved performance, therefore the inclusion of sufficient protein at the right time is recommended to strength and endurance athletes as part of an optimal diet (Tipton *et al.*, 2007:21; Rodriguez *et al.*, 2009:509; Witard *et al.*, 2014:86).

According to current literature the protein requirement for sedentary individuals is 0.8 - 0.9g/kg body weight per day (Phillips, 2004:689). A number of studies have indicated that intense training increase athletes' protein requirement (van Loon, 2014:106; Kreider *et al.*, 2010:9), and therefore protein guidelines specifically for athletes and active individuals have been developed and are available in the literature (Phillips & van Loon, 2011:31; Kreider *et al.*, 2010:9). The International Olympic Committee (IOC) recommends a protein intake of 1.3 - 1.8g/kg body weight per day for athletes competing in resistance and endurance training (Phillips & van Loon, 2011:31). The American College of Sports Medicine (ACSM) recommendations are very similar to the IOC (1.2 - 1.7g/kg) (Rodriguez *et al.*, 2009:515) whilst the International Society of Sports Nutrition (ISSN) recommends a slightly higher intake (1.5 - 2.0g/kg body weight per day) for individuals taking part in intense or high volume training (Kreider *et al.*, 2010:9).

Optimal protein ingestion involves more than just the quality and quantity of protein. The timing of protein intake in relation to exercise (i.e. before, during or after exercise) is also an important consideration for athletes (Schoenfeld *et al.*, 2013:53; Witard & Tipton, 2014:10). For example, protein intake after exercise has shown to maximise muscle protein synthesis (Phillips & van Loon, 2011:32). Repeated bouts of increased muscle protein synthesis can further result in increased muscle size and muscle strength (Witard & Tipton, 2014:11). Kerksick *et al.* (2008:9) has demonstrated that the ingestion of 6 - 20g essential amino acids (EAAs) with carbohydrates immediately to three hours after an exercise bout has shown to significantly stimulate muscle

protein synthesis compared to when non-essential amino acids (NEAA) and no protein was added to the recovery meal. In fact, the IOC, ISSN and the ACSM all agree that the ingestion of ~20g of high quality protein within 30 minutes post-exercise (also referred to as the “window of opportunity”) has a beneficial effect on muscle protein synthesis (Potgieter, 2013:11).

Rugby players require aerobic and anaerobic qualities, but also muscular strength, power and speed to perform optimally in their sport (Duthie *et al.*, 2006:202). Sound nutrition is essential to provide them with energy and to optimise muscle mass to enhance speed, strength and power (Duthie *et al.*, 2006:202). Due to the nature of the game and specifically the fact that rugby is a contact sport, rugby players not only require, but can also benefit from additional protein to optimise muscle mass and muscle strength, repair muscle damage and help glycogen restoration following exercise (Kimiwiwe & Simiyu, 2009:1307). In South Africa rugby is regarded as a national sport that is being developed from a young age. The pressure even for young rugby players to perform is evident from events such as the high school Coca-Cola Craven week, a prestigious well sponsored tournament that is organized only for the best high school teams in the country (Hendricks *et al.*, 2015:558). In universities, rugby players are further being moulded, developed and prepared for the professional league and they often experience even more pressure to compete at top level since they are eligible to be selected for a provincial and even national under 21 or senior Springbok team (McMillan, 1997:8; Hale, 2013:3). Training and competition in addition to academic commitments provide unique challenges to university rugby players (Simiyu, 2010:17), not only in terms of optimal time management, but also in terms of optimal nutrition.

A number of studies on rugby players including South African university rugby players and American college football players have examined dietary intakes during training and competition (Imamura *et al.*, 2013:2; Kirwan *et al.*, 2012:1; Lundy *et al.*, 2006:199; Potgieter *et al.*, 2014:35). Potgieter *et al.* (2014:35) examined the habitual and match-day dietary intakes in South African university rugby players and reported an overall mean habitual protein intake of 2.4 ± 0.7 g/kg body weight (BW) per day, much higher compared to the recommended intake of 1.2 - 1.7 g/kg BW per day. The protein content of the pre-event meal (1.2 ± 0.6 g/kg BW) and post-event meal (0.9 ± 0.4 g/kg BW) on match day were also higher than the recommendations (0.15 - 0.25 g/kg and 0.2 - 0.5 g/kg BW respectively). Burkhart, (2010:11) determined the nutrition knowledge in talented adolescent athletes including rugby players and reported that rugby players had a poor sports nutrition knowledge ($42.2\% \pm 0.5$). They specifically indicated poor knowledge regarding the amount and source of pre-event food intake (0%), during event food and fluid intake (17.9%) and the amount of recovery food (14.3%). Walsh *et al.* (2011:371) also indicated that out of a group of 203 Irish schoolboy rugby players, 56% of the players perceived protein to increase strength, facilitate improved training (25%), promotes weight gain (muscle mass) (63%) and aid

recovery (56%). Although there are data available on nutritional knowledge of rugby players (Alaunyte *et al.*, 2015, Hale, 2013, Walsh *et al.*, 2011; Burkhart, 2010) very little is known on university rugby players' knowledge specifically regarding the timing of protein intake.

Information on the knowledge and perception of timing of protein intake of university rugby players can be useful since nutrition knowledge and perceptions have been shown to influence athletes' nutritional practices. Therefore through examining their knowledge and perceptions, areas of poor knowledge can be identified and improved through education (Walsh *et al.*, 2013:371; Strachan *et al.*, 2009:51). University athletes are a susceptible target audience for nutrition education given that they find themselves in a learning environment. Furthermore, students are mostly in a phase of transition and thus have the potential to be influenced (Barzegari *et al.*, 2011:1012). Accurate knowledge assessment in university athletes requires a valid and reliable tool to collect knowledge data (Whati *et al.*, 2005:77). To our knowledge, a valid and reliable questionnaire to specifically determine the knowledge of protein timing in university athletes is also not available from the literature. Therefore this Masters dissertation aims to examine the knowledge and perceptions of university rugby players on the timing of protein ingestion.

1.2 Aims and objectives

The aim of the dissertation is to determine the knowledge and perceptions of North-West University rugby players on timing of protein intake.

The objectives of the dissertation are to:

1. Develop and validate a knowledge questionnaire on timing of protein intake.
2. Determine the knowledge of university rugby players on timing of protein intake.
3. Examine the perceptions of university rugby players on timing of protein intake.

1.3 Research team

The table on the next page provides a summary of the research team, including the specific role and contribution of each team member towards this MSc dissertation.

Table 1.1: Research team

Team member	Affiliation	Role and contribution
Dr L Havemann-Nel (PhD. Exercise Science BDietetics)	Centre of Excellence for Nutrition (CEN), North-West University(NWU), Potchefstroom Campus	Supervisor of the MSc dissertation. Guidance regarding writing the protocol and ethics application, development of questionnaire, writing of the literature review, overview of data collection, assistance with statistical analysis, interpretation of results and writing up of data.
Dr CR Botha-Ravyse (PhD. Biokinetics)	Centre of Excellence for Nutrition (CEN), North-West University(NWU), Potchefstroom Campus	Co-supervisor of the MSc dissertation, guidance regarding writing the protocol, conducting focus group discussions, analysis and interpretation of qualitative data and writing up of the data.
Dr MJ Lombard (PhD. Nutrition and Dietetics BSc Dietetics)	Centre of Excellence for Nutrition (CEN), North-West University, Potchefstroom Campus	Assistant-supervisor of the MSc dissertation, guidance regarding the focus group discussions and validation of the knowledge questionnaire.
Ms L Swanepoel (BSc. MBW and Nutrition Honn. Nutrition)	MSc student at Centre of Excellence for Nutrition (CEN), North-West University(NWU), Potchefstroom Campus	Full-time MSc student. Writing of the protocol, ethics application and literature review. Also involved in questionnaire development, quantitative data collection, analysis and interpretation of data, and writing up the data and final MSc dissertation.

1.4 Structure of dissertation

This MSc dissertation is in article format and is presented in six chapters. Chapter one provides a short rationale for the study, outlines the aim, objectives and hypothesis, and gives an overview of the research team and structure of the dissertation. Chapter two presents the literature review where the researcher provides a brief overview of the role and importance of dietary protein in general and expanding on the specific role and importance of protein for athletes. The literature review continues to discuss rugby as a sport and the role of nutrition and particularly protein in rugby players. The timing of protein intake is discussed and a summary of previous studies that have examined the effect of protein timing on various outcomes is provided. The literature review also examines the knowledge and perception of athletes, in particular college or university athletes on timing of protein intake. Chapter three includes the first research article entitled: "Development, validation and reliability of a questionnaire to determine the knowledge of athletes on the timing of protein ingestion". This article is written according to the specifications of the Journal of Nutrition Education and Behavior and has been submitted to the Journal of Nutrition Education and Behavior (article is still in review). For the purpose of the dissertation however, the tables and figures have been included in the text for convenience. The line spacing has also been reduced to save space and printing costs.

Chapter four includes the second article entitled: “Knowledge and perceptions of North-West university rugby players on the timing of protein ingestion”. This article will be submitted to the International Journal of Sports Nutrition and Exercise Metabolism and is written according to the journal specifications. In chapter five, the researcher provides a short summary and conclusion of the most relevant and important findings of the MSc as a whole, acknowledges the limitations and makes recommendations based on the findings. The final chapter provides the bibliography for the references cited in Chapters one, two and five. These references are according to the North-West University Harvard style.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Over the past 20 years, researchers have documented how optimal nutrition benefits exercise performance (Kreider *et al.*, 2010:7). The importance of protein in particular has long been recognized. From coaches of Olympians in ancient Greece to today's multi-millionaire athletes, have considered protein a key nutritional component for athletic success (Tipton & Wolfe, 2004:65). Protein is considered an important nutritional factor not only for athletes competing in strength and power sports, but also for endurance athletes (Tipton *et al.*, 2007:21; Phillips & van Loon, 2011:31).

The idea that athletes need a high protein diet has received attention, and indeed there is evidence to suggest that the requirement for protein is increased by physical activity (Phillips & van Loon, 2011:30). Athletes involved in intense training have higher dietary protein needs than individuals who do not train (Tipton *et al.*, 2007:22). According to current literature the protein requirement for sedentary individuals is 0.8 - 0.9g/kg body weight per day (Phillips, 2004:689). The International Olympic Committee (IOC) recommends a protein intake of 1.3 - 1.8g/kg body weight per day for athletes competing in resistance and endurance training (Phillips & van Loon, 2011:31). The International Society of Sports Nutrition (ISSN) recommends an even a higher intake (1.5 - 2.0g/kg body weight per day) for individuals taking part in intense or high volume training (Kreider *et al.*, 2010:9).

Protein plays an important role in increasing strength, maximizing protein synthesis, increasing glycogen resynthesis, repairing muscle damage, promoting training adaptations, enhancing nitrogen retention and enhancing body composition in the long term (Cermak *et al.*, 2012:1454). These benefits may then translate into an improved performance, and sufficient protein is therefore recommended to strength and endurance athletes as part of a balanced habitual and optimal sports diet (Tipton *et al.*, 2007:21; Witard *et al.*, 2014:86).

Optimal protein ingestion involves more than just the quantity and quality of protein. The timing of protein intake in relation to exercise (i.e. before, during or after exercise and competition) also has an impact on strength and power athletes (Schoenfeld *et al.*, 2013:53; Witard & Tipton, 2014:10). For example, protein intake after exercise has shown to maximise muscle protein synthesis, and repeated bouts of increased muscle protein synthesis results in increase muscle size and muscle strength (Witard *et al.*, 2014:93). Kerksick *et al.* (2008:9) has demonstrated that the ingestion of 6 - 20g essential amino acids (EAAs) with carbohydrates (CHO) immediately after an exercise session has shown to significantly stimulate muscle protein synthesis compared to when no protein was added to the recovery meal. In fact, the IOC, ISSN and the

American College of Sport Medicine (ACSM) agree that the ingestion of ~20g high quality protein within 30 minutes after exercise (also referred to as the “window of opportunity”) has a beneficial effect on muscle protein synthesis (Potgieter, 2013:11).

Rugby players require aerobic and anaerobic qualities, but also muscular strength, power and speed (Duthie *et al.*, 2006:202). Sound nutrition is essential to provide them with energy and to optimise muscle mass to enhance speed, strength and power (Duthie *et al.*, 2006:202). Due to the nature of the sport, rugby players not only require, but can also benefit from additional protein to optimise muscle strength for the contact nature of the game, to repair muscle damage and to help glycogen restoration following exercise (Kimiwiwe & Simiyu, 2009:1307). In South Africa rugby is regarded as a national sport and is being developed from school level. Competition is intense at university level and nutritional status can separate winning from losing (Strachan *et al.*, 2009:2). University rugby players who are being moulded, developed and prepared for the professional league, experience extreme pressure to compete at top level (Hofmann *et al.*, 2007:85). University rugby players are not only subjected to unique pressures to perform optimally in their sport, they often pursue academic and semi-professional sporting careers simultaneously which is demanding, stressful and highly competitive (Hale, 2013:10). The discipline of sports nutrition is expanding therefore adequate nutrition knowledge among athletes becomes more important to stay on top of their sport (Hale, 2013:11). However, athletes often demonstrate poor nutritional knowledge (Walsh *et al.*, 2011:365) resulting in poor nutritional choices and practices. Since knowledge can influence what and when athletes eat (Dunn *et al.*, 2007:5), improving nutritional practices, including protein timing practices via education will be useful in optimizing performance (Kreider *et al.*, 2010:11).

Limited research has however been conducted on nutritional knowledge of athletes, especially regarding the athletes’ knowledge on timing of protein intake. An investigation regarding the challenges of being a collegiate student athlete, demonstrated that these athletes experience unique pressures balancing academics and athletics careers, experience a lack in social life on campus and also deal with large stress loads (Göktas, 2010:54). University athletes are a susceptible target audience for nutrition education given that they find themselves in a learning environment. Furthermore, students are mostly in a phase of transition and thus have the potential to be influenced (Barzegari *et al.*, 2011:1012).

This literature review will provide the reader with a brief overview of the role of protein in the body as well as the process of protein digestion. The review will further expand on the role and benefits of protein specifically in exercise. Finally the review will aim to provide an overview of studies that have explored the knowledge and perceptions of protein, with a specific reference to timing of protein intake in athletes, in particular university rugby players.

2.2 Protein as macronutrient

2.2.1 Definition of protein

The word 'protein' is derived from the Greek word 'proteos' which means primary or 'most important' (Whitford, 2013:2). Protein is an essential nutrient for life. After water, protein is the most abundant substance in the body and found in every body part including skin, muscle, organs, bone, hair and nails. Proteins are large, complex molecules made up of thousands of smaller units called amino acids (AA), which attach to one another to form long chains. These protein chains of AA fold into three-dimensional shapes, and the sequence of AA determine each protein's structure and its function (Whitney & Rolfes, 2012:170). Proteins are comprised of 20 AA, nine of which are considered essential amino acids (EAA) (including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) and 11 that are non-essential amino acids (NEAA) (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine) (Tarnopolsky, 2003:67). The essential AA can't be synthesized by the body and must be ingested through the diet.

Protein sources are rated according to the amount of EAA they provide: A complete protein source provides all of the EAA and are also called high quality proteins. Animal-based foods for example meat, poultry, fish, milk, eggs and cheese are considered complete or high quality protein sources (Hulmi *et al.*, 2010:51). An incomplete protein source is one that is low in one or more of the EAA. Complementary proteins are two or more incomplete protein sources that together provide adequate amounts of all the EAA (Zieve, 2009:1).

2.2.2 Overview of roles of protein

Proteins play critical roles in the body including being the most important building blocks for muscles, cell membranes, connective tissue, hormones, enzymes and the immune system (Whitney & Rolfes, 2011:170). Protein also aid in maintaining acid-base and fluid balance, transporting nutrients and can provide a source of energy when necessary (Hall, 2010:833). Without adequate amounts of protein in the body vital body functions like breathing, fighting infections and maintaining organs would not be possible. Proteins can be described according to their large range of functions in the body and the most important functions of protein are summarized in Table 2.1 (Whitney & Rolfes, 2011:175).

Table 2.1: Roles of protein in the body

Function	Description
Structural and mechanical support and maintenance	Proteins are the body's building materials, providing strength and flexibility to tissues, tendons, ligaments, muscles, organs, bones, nails, hair, and skin. Proteins are needed for on-going maintenance.
Enzymes and hormones	Proteins are needed to make most enzymes that speed up reactions in the body and hormones that direct specific activities, such as regulating blood glucose level.
Fluid balance	Proteins play a major role in ensuring that body fluids are evenly dispersed in the blood and in cells.
Acid-base balance	Proteins act as buffers to help keep body fluid pH balanced within a tight range. A drop in pH will cause body fluids to become too acidic, whereas a rise in pH can make them too basic.
Transport	Proteins transport oxygen, waste products, and nutrients through the blood and into and out of cells.
Antibodies and the immune response	Proteins create specialized antibodies that attack harmful pathogens in the body.
Energy	Because proteins provide 4 calories per gram, it can be used as fuel or energy in the body.

Table adapted from Whitney & Rolfes, (2011:175).

2.2.3 Protein digestion and turnover

Figure 2.1 provides a schematic overview of protein digestion in the body. Protein digestion in the stomach starts with stomach hydrochloric acid (HCL) untangling the bonds of protein strands that were ingested via diet. The digestive enzyme pepsin, produced in the stomach lining, is activated by the stomach's acidic environment to break down protein to shorter fragments for absorption into the small intestine (Hall, 2010:833). In the small intestine, enzymes like proteases and peptidases further break down the strands to even smaller polypeptides. The protein fragments are absorbed into cells of the small intestine lining, where these fragments are broken down into single AAs, which enter the blood and travel to the liver.

The liver uses these AA depending on the body's needs. For example, AA may be used to synthesize new protein or, if necessary AA can be converted to glucose that can be used as energy if CHO intake through diet is low. A number of AA are also stored in a free amino acid pool (Whitney & Rolfes, 2012:172). AA in excess to what is required by the body and stored in the amino acid pools are degraded completely and excreted (Whitney and Rolfes, 2011:177). In this breakdown process, amino acids lose their amine groups. Nitrogen in the amine group's forms ammonia (NH₂), which can be toxic to cells in high amounts. The liver converts the NH₂ to

urea, a waste product that is excreted in urine via the kidneys. The carbon-containing remains of the AAs are converted to glucose, used as energy, or stored as fat.

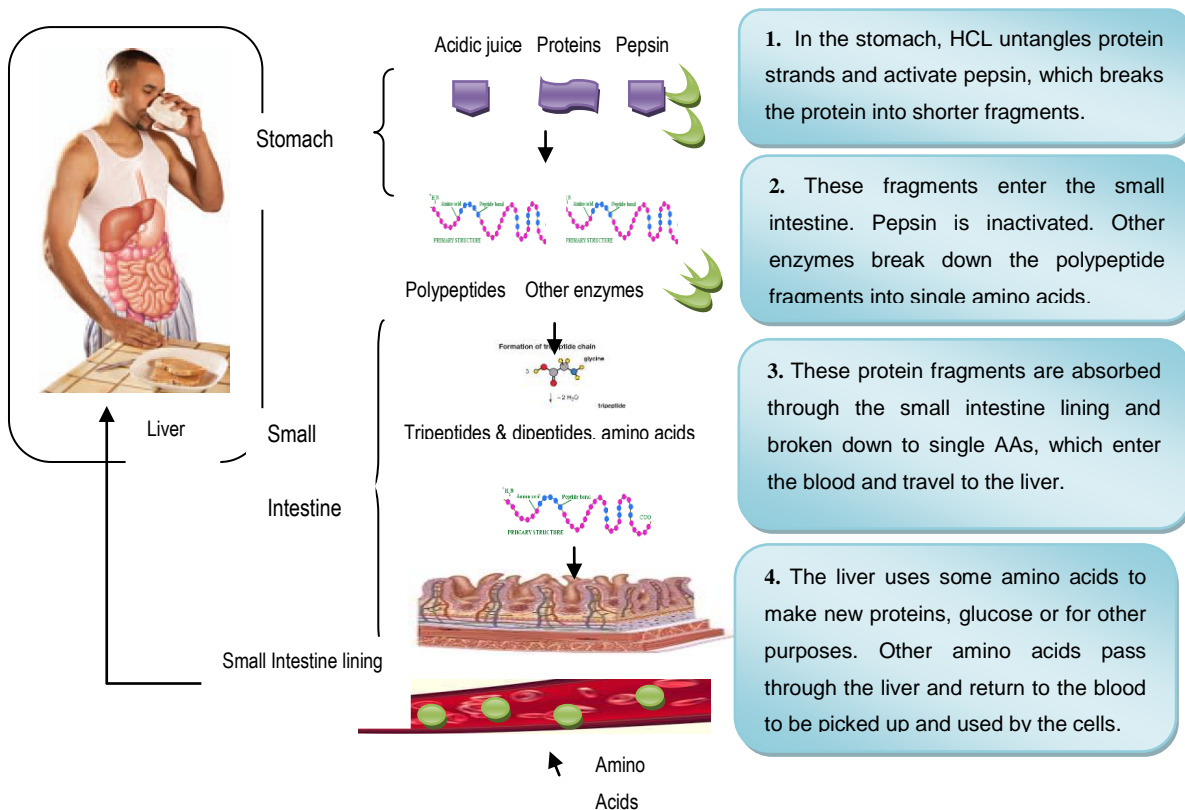


Figure 2.1: Summary of the protein digestion process

The body is constantly and simultaneously synthesising muscle protein and degrading or breaking down muscle protein by either supplying AAs – the ‘building blocks’ of protein – to the free amino acid pool or extracting AAs from the free amino acid pool in the body throughout the day (Poortmans *et al.*, 2012:879). This process of degrading and synthesizing protein is called protein turnover (Poortmans *et al.*, 2012:879). Proteins and AAs are lost daily through skin, hair, and nails therefore dietary protein intake is vital to replace these losses. Exercise-induced AA oxidation also contributes to increase losses (Poortmans *et al.*, 2012:880). As mentioned before, the body cannot synthesize EAA, so dietary protein to replace especially the EAA is important (Di Pasquale, 2007:65).

2.3 Protein and exercise

2.3.1 Roles of protein in exercise

Protein plays an important role in the body’s adaptation in response to exercise (Tipton & Wolfe, 2004:65). Protein and more specifically AA primarily form the building blocks to repair and manufacture new tissue including muscle following exercise. AAs also serve as building blocks

for hormones and enzymes that regulate metabolism and other functions during and after exercise. Protein/AA can furthermore provide a small source of fuel for the exercising muscle (Tipton & Wolfe, 2004:67) and optimizes glycogen repletion following exercise (Hoffman *et al.*, 2007:85).

2.3.1.1 Promoting training adaptations

Different athletes consume protein for different reasons (Tome & Bos, 2000:1868). Protein serves as substrate and trigger to facilitate training adaptations in response to both resistance and endurance exercise. Resistance/power sport athletes, including team sport athletes such as rugby players mainly consume protein to optimize muscle protein synthesis (specifically increased myofibrillar protein synthesis) and achieve a net positive protein balance with the ultimate aim to increase muscle mass, strength and power (Tipton & Wolfe, 2004:66). Endurance sport athletes like long distance runners, cyclists and also team athletes like rugby (endurance component), mainly consume protein for increased mitochondrial protein synthesis with the aim to enhance oxidative capacity and to optimize muscle damage recovery (Tipton & Wolfe, 2004:67). Protein consumption therefore enhances MPS rates and possibly lower MPB, thus improving net protein balance. This improvement appears to accumulate to promote greater protein retention in the case of resistance exercise and may change training induced adaptations during endurance exercise. If protein is consumed close to exercise, better adaptations are promoted (i.e. greater muscle mass gain or greater gains in oxidative capacity) (Phillips & van Loon, 2011:30).

2.3.1.2 Maximizing protein synthesis and hypertrophy

Maximizing muscle protein synthesis (MPS) with the aim to facilitate hypertrophy (increased muscle mass) is a common and important nutritional goal particularly for athletes competing in power/strength sports, including rugby (Phillips, 2006:647). Muscle hypertrophy takes place when MPS repeatedly exceeds muscle protein breakdown (MPB) (Tipton *et al.*, 2007:28). If MPS exceeds MPB, a positive net protein balance is achieved and repeated periods of positive protein balance will result in increased muscle mass/hypertrophy (Hoffman *et al.*, 2009:182). A number of factors can stimulate MPS without necessarily promoting a positive net protein balance including an exercise stimulus (bout of resistance training) (Willoughby *et al.*, 2007:467) or consuming protein, which supply AAs to stimulate MPS (Ivy, 2004:132). Simply eating food containing CHO, which result in the hormone insulin being secreted can also stimulates MPS (Rennie & Tipton, 2006:476). MPS can therefore take place with and without protein, but MPS will not necessarily exceed MPB. In order to promote hypertrophy, protein consumption is essential. To effectively and optimally stimulate hypertrophy, a source of protein, a training

stimulus and sufficient CHO energy are needed (Moore *et al.*, 2009:897; Rennie & Tipton, 2006:476).

2.3.1.3 Repair and recovery

Professional athletes often train twice daily or compete/train again 24 hours after an exercise session. Attention to optimal recovery, including muscle function recovery is important during this time frame. Recovery from exercise is a complex process requiring replacement of body's fuel stores, initiation of training adaptations and repair of damaged muscle tissue. During exhausting exercise (resistance exercise and endurance exercise) damage occur to the active muscles resulting in continued increased rates of protein degradation following exercise (Rennie & Tipton, 2000:460). The ingestion of protein has shown to stimulate muscle protein synthesis resulting in positive net whole body protein balance following endurance exercise (Howarth *et al.*, 2009:1394) and resistance exercise. A positive net protein balance is not only required to promote muscle hypertrophy as discussed above, but also to enhance recovery through the repair and remodelling of damaged proteins (Ivy *et al.*, 2004:134).

More recently Howatson *et al.* (2012:20) have reported a reduction in muscle damage, prevention of force reduction and enhanced recovery from resistance exercise in individuals consuming AAs after training. The co-ingestion of protein with CHO after exercise has also shown to reduce plasma creatine kinase (CK), a reported marker of muscle soreness. Ivy *et al.* (2004:132) have further demonstrated that lower CK concentrations and lower ratings of muscle soreness accompany improvements in the recovery of time-to exhaustion.

2.3.1.4 Increasing glycogen re-synthesis

The major fuel source used by the skeletal muscles during prolonged exercise is muscle glycogen. Research has shown endurance is directly related to the initial muscle glycogen stores (Ivy *et al.*, 2001:236). When these stores are depleted strenuous exercise cannot be maintained and perception of fatigue during prolonged intense exercise increase as muscle glycogen decline (Ivy, 2004:133). Therefore due to the importance of muscle glycogen for sustained endurance exercise, considerable research has been conducted to establish efficient ways to replenish muscle glycogen stores following exercise, especially when recovery time is limited (e.g. in the case of events or tournaments consisting of consecutive days of competition or training). The ingestion of high glycaemic CHO at a rate of at least 1g/kg/hour following exercise for glycogen resynthesis is recommended. However, if sufficient CHO is not available (<1g/kg), the addition of protein to CHO have shown to enhance glycogen resynthesis to the same extend and even greater extends compared to when CHO were consumed in higher amounts (Rodriquez *et al.*, 2009:514). Ivy *et al.* (2002:1343) have demonstrated significantly

greater muscle glycogen restoration rates (88.8 ± 4.4 mmol/l) 4-hours after the ingestion of 80g CHO in combination with 28g protein compared to the ingestion of only 80g CHO (70.0 ± 4.0 mmol/l) as well as an iso-caloric amount of 108g CHO (75.5 ± 2.8 mmol/l). Of greater interest was the immense difference in glycogen storage between the treatments during the first 40 minutes of recovery. Glycogen storage was twice as fast after CHO and protein treatment as after high CHO treatments, and four times faster than after low CHO treatment (Ivy *et al.*, 2002:1343). The results indicate that co-ingestion of protein and carbohydrate increases the efficiency of muscle glycogen storage when supplementing at intervals greater than one hour apart (Ivy *et al.*, 2002:1343).

2.3.1.5 Role as hormones and enzymes

Hormones control many of the body's physiological processes including gene regulation and metabolism. Metabolically, hormones regulate the synthesis and breakdown of proteins, carbohydrates, and fats and can therefore impact the formation of lean mass in the long term (Volek, 2004:692). Exercise, specifically resistance exercise prompts acute physiological responses and chronic adaptations that are critical for increasing muscular strength, power, hypertrophy and muscular endurance (Kreamer *et al.*, 2007:644). Protein intake has been suggested to influence the anabolic hormones involved with muscle remodelling (Burd *et al.*, 2009:570). A greater anabolic hormone response could have significant impacts on the repair and recovery of skeletal muscle after resistance exercise sessions and play a vital role in the muscle remodelling (Kreamer *et al.*, 2007:645). High protein diets have been associated with changing resting concentrations of testosterone, cortisol and insulin-like growth factor and increases in resting growth hormone levels have been noted (Hofmann *et al.*, 2007:85; Kreamer *et al.*, 2007:637; Volek, 2004:692).

- **Testosterone**

Testosterone has potent anabolic effects on muscle tissue. Protein consumption before a workout may result in a decreased secretion rate or an increased metabolic clearance rate of testosterone (Hulmi *et al.*, 2010:51). It has also been suggested that pre-exercise protein intake may lead to an increase in testosterone uptake by cells during exercise (Volek *et al.*, 2004:693). An acute testosterone response to resistance exercise appears to be influenced by nutrition. These mechanisms may contribute to reduce the magnitude of total testosterone responses and perhaps also explain the free testosterone response during immediate and post-exercise recovery periods (Hofmann *et al.*, 2008:85).

- **Growth Hormone**

Growth hormone (GH) increases muscle and skeletal growth while subsequently increasing protein synthesis, lipolysis, and glucose conservation (Volek, 2004:692). Studies have shown

that protein have an effect on regulation of GH secretion. Ingestion of large doses of certain amino acids (arginine, lysine, and ornithine) can increase GH levels (van Loon *et al.*, 2000:106). Volek, (2002:864) reported that a protein and carbohydrate supplement consumed before and immediately after resistance exercise enhanced the acute GH response from 0 - 30 minutes post-exercise compared with a non-caloric placebo despite similar glucose levels (Volek, 2002:864). Also a protein and carbohydrate supplement consumed immediately and 120 min after resistance exercise increased GH during late recovery when glucose levels were lower (Volek, 2004:692).

- **Insulin**

Insulin is one of the most anabolic hormones in the body. In terms of protein metabolism, insulin promotes glucose uptake, glycogen formation, and protein synthesis in the presence of sufficient amino acids. Certain amino acids can increase insulin, therefore interest was shown to combine protein with carbohydrate to maximize insulin secretion, in the hopes to enhance post-exercise glycogen resynthesize (van Loon *et al.*, 2000:107) and protein anabolism (Rasmussen *et al.*, 2000:386; Tipton *et al.*, 2001:204). Enhanced insulin levels resulting from CHO and protein ingestion are expected to have a positive effect on NBAL because insulin is generally accepted as a stimulator of protein synthesis when adequate amino acids are available (van Loon *et al.*, 2000:106). Following exercise, increased plasma insulin levels are key to limiting muscle damage. Consuming protein prior to exercise has shown to increase insulin concentrations during the post-exercise period (Hulmi *et al.*, 2010). Studies from van Loon *et al.* (2000:106) and Williams, (2003:63) has shown to improved performance as result of insulin stimulation, which has been observed with post-exercise CHO and protein feedings (van Loon *et al.*, 2000:106; Williams, 2003:63).

- **Cortisol**

Vigorous exercise results in increased cortisol secretion (Di Pasquale, 2007:51), while amino acids ingestion causes a desirable reduction in cortisol concentration during exercise (Bird *et al.*, 2006:225). In addition to prompting breakdown of protein, cortisol has been shown to prevent the BCAA-induced anabolic shift in protein balance and inhibits BCAA action on the phosphorylation of protein in the pathway, thus preventing increased muscle protein synthesis (Bird *et al.*, 2006:226). Therefore a reduction in plasma cortisol responses, through amino acid consumption, may lessen the catabolic effect observed during prolonged exercise and allow greater potential for protein synthesis (Hoffman *et al.*, 2007:85).

2.3.1.6 Energy substrate

Although protein is not a primary metabolic fuel that is oxidized during exercise but rather serve as structural component to increase muscle mass and functional strength in response to

exercise, a number of the AAs, specifically the BCAAs can be oxidized during exercise to provide fuel (Rennie *et al.*, 2006:264). In fact, endurance exercise is associated with marked increases in specifically leucine oxidation (Rennie & Tipton, 2006:476). Generally only a small percentage (<5% of total energy) of protein is used as a source of energy (Meltzer, 2011:45), but certain conditions can increase the reliance on protein for energy including training at high altitude and limited CHO and fat availability (Egan & Zierath, 2013:166). Insufficient CHO intake before and during exercise restricts muscle glycogen and blood glucose energy availability and results in gluconeogenesis (conversion of AA to glucose for fuel) (Borsheim *et al.*, 2002:648).

2.3.2 Protein requirements

2.3.2.1 Quantity of protein

The idea that a diet higher in protein is necessary for athletes is appealing and indeed evident. The requirement for protein is increased by physical activity (Phillips, 2006:647). General fitness activities can elevate protein requirements to 1.0 g/kg body weight/day and higher (Phillips & van Loon, 2011:29). Athletes involved in intense training have even higher dietary protein needs (Tipton & Witard, 2007:17). It is important to identify whether physical activity involves endurance exercise such as running, in which amino acids are oxidized, or resistance exercise aimed to achieve muscle hypertrophy. This will determine dietary protein requirements (Phillips, 2006:647; Slater & Phillips, 2011:320).

A groundbreaking study where endurance runners were compared to sedentary individuals suggested that endurance athletes require 1.67 times more daily protein than sedentary individuals (Tarnopolsky *et al.*, 1998:890). Investigations on whole-body protein turnover and skeletal muscle syntheses rates in trained endurance humans however suggested that a protein intake of 1.2 g/24 h should achieve a positive NBAL (Williams *et al.*, 2004:63).

Relative to endurance athletes and the sedentary population, a greater protein need exists for strength/power athletes (Willoughby *et al.* 2007:467). Literature support that protein contributes to metabolism even during single sessions of high-intensity exercise and that training influence the content of enzymes involved in protein metabolism (Howarth *et al.*, 2007:1394). A single session of resistance exercise stimulates gene expression related to protein synthesis (Hulmi *et al.*, 2009:52). For strength trained individuals to maintain a positive nitrogen balance it appears that daily protein consumption should be between 1.6 - 1.8 g/kg/day (Tarnopolsky *et al.*, 2004:662). The greater protein requirement is related to the enhanced protein synthesis necessary to assist in the repair and remodeling process of skeletal muscle fibers damaged during a resistance exercise session (Kraemer *et al.*, 2007;637 Ratamess *et al.*, 2003:250). This is important for improving both muscle size and strength. The beneficial effects of a high

protein intake may also be reflected by improvements in body composition through increasing lean tissue (Cribb & Hayes, 2008:2).

2.3.2.2 Quality of protein

High-quality protein is needed to increase muscle mass (Burke *et al.*, 2012:452; Koopman *et al.*, 2009:712). According to the protein digestibility corrected amino acid score (PDCAAS) index, which is the most commonly accepted and understood index to measure protein quality, a number of proteins are classified as high quality (Kreider & Campbell, 2009:13). This means that these proteins, which include milk and the constituent proteins of milk – casein and whey – egg, isolated soy protein and most meats, have a PDCAAS score of 1.0 or close to 1.0 (Hoffman & Falvo, 2004:119). These sources of protein have high concentrations of EAAs (Tipton *et al.*, 2004:2075). Research has shown EAAs are needed for muscle protein synthesis. EAAs can only be obtained through the diet since the body cannot synthesise them. The BCAA (all EAAs) and, specifically, leucine are very important since these AAs acts as signals to activate important processes in MPS (Moore *et al.*, 2009:897; Tang *et al.*, 2007:1132).

Bohé *et al.* (2003:315) demonstrated that human MPS is modulated by the extracellular (blood concentration) availability of EAA, and several publications have confirmed this observation following resistance exercise (Dreyer *et al.*, 2009:392; Tang *et al.*, 2007:1133). Borsheim *et al.* (2002:648) showed that 6g of mixed AAs elevated protein synthesis after exercise. In the same experiment, 6g of EAAs doubled protein synthesis, concluding that NEAAs were not required to promote protein synthesis. EAAs content seems to be the important component (Hulmi *et al.*, 2010:51). Leucine along with isoleucine and valine, are BCAA which is considered the most anabolic amino acids. Leucine may be the most important AA for stimulation of muscle protein synthesis (Hulmi *et al.*, 2010:51; Di Pasquale, 2007:2) due to substrate provision.

Milk proteins are high in leucine with whey having the highest concentration of leucine (Wilkinson *et al.*, 2007:1031). Casein is also high in leucine, but since casein clots in the stomach, casein is digested slower and the rate of leucine appearance is slower. Although isolated soy has a lower concentration of leucine compared with casein, the rate of leucine appearance is faster and, therefore, isolated soy protein is more effective than casein to stimulate MPS (Di Pasquale *et al.*, 2000:13).

Skimmed milk supplementation (± 18 g protein) has been proposed to athletes after resistance exercise (Wilkinson *et al.*, 2007:1031) indicating a greater lean mass accretion and functional performance after training. Whey proteins, or whey components, have also been proposed as they contain nearly 50% of the EAA and about 26% of the BCAA (Dreyer *et al.*, 2009:392). Different research teams used whey proteins to supplement athletes after resistance exercise to

promote MPS (Moore *et al.*, 2009:897; Tang *et al.*, 2007:1132). These publications concluded that whey protein taken immediately after resistance exercise stimulates MPS, and more specifically the myofibrillar protein fraction, for up to at least 6 h after exercise (Moore *et al.*, 2009:901). The growing interest in the potential of bovine milk as an exercise beverage during recovery from resistance exercise has proven to be an effective post-exercise beverage that results in favourable alterations in protein metabolism. Milk consumption acutely increase muscle protein synthesis, leading to improved net muscle protein balance. Furthermore, when resistance training is combined with post exercise milk a greater increase in muscle hypertrophy and lean mass was observed. Milk is also a nutrient dense beverage that is safe and effective (Roy, 2008:1).

Egg protein is obtained from chicken egg whites or whole eggs. The PDCAAS of egg protein is similar to milk protein (Kreider & Campbell, 2009:13). A number of studies have evaluated the effect of egg protein on nitrogen retention and physiological adaptations to exercise in comparison to other proteins. Results of these studies indicate that egg protein is as effective as milk in promoting nitrogen retention (Kreider & Campbell, 2009:15). Soy lacks the EAA methionine and is therefore not considered a complete protein. However, soy has a relative high concentration of remaining essential amino acids and is therefore considered a high quality protein (Kreider & Campbell, 2009:15). The PDCAAS of soy is similar to dietary meat and fish and slightly lower than egg and milk protein. Consequently soy serves as excellent source of protein, particularly for vegetarians. Research also indicates several potential health benefits since soy beans are low in fat and cholesterol and a good source of protein (Jenkins *et al.*, 2010:230). A practical and affordable dietary protein source to consume following exercise is flavoured low fat milk that provides a source of high-quality protein and CHO and has shown to stimulate MPS (Phillips & van Loon, 2011:31).

2.3.2.3 Timing of protein intake

A recent area of focus in studies examining protein is the area of *timing of protein* consumption or protein timing. Protein timing is an effective strategy designed to optimize the adaptive response to both resistance and endurance exercise (Phillips & van Loon, 2011:32). This strategy involves consuming protein mostly in and around a resistance training session (e.g. pre, during or after resistance training) to facilitate muscular repair and remodeling, and thereby enhance post-exercise strength and hypertrophy-related adaptations (Kerksick *et al.*, 2008:9). Studies examining the acute effect of protein ingestion have demonstrated that ingestion occurring close to a resistance workout (immediately before and/or within an hour post-exercise) significantly enhances MPS rate and muscle protein accretion compared to delayed ingestion (Kerksick *et al.*, 2008:5). The stimulus of resistance exercise opens a muscle protein synthesis “window of opportunity” that is complementary with the provision of other stimulatory factors,

such as AA (Phillips, 2012:161). A number of studies have also shown that protein ingestion during and after endurance exercise may be beneficial.

Table 2.2 provides a summary of studies examining the effect of protein consumption during or after resistance exercise. Protein consumption during and after resistance exercise provide a potent stimulus for muscle protein synthesis. Ingesting protein during resistance exercise has shown to counterbalance muscle damage and facilitate greater training adaptations after prolonged periods of resistance training (Burd *et al.*, 2011:225).

Table 2.2: Effect of protein consumed before, during and after resistance training

Reference	Subjects	Intervention	Results
Areta <i>et al.</i> (2013)	24 young, healthy trained males with at least 2 years of high-intensity RE experience (training ≥ 2 times per week) Parallel design (3 groups of 8)	Effect of 80g whey protein throughout 12h recovery period following a bout of RE on MPS A: 8x10g every 1.5h (PULSE) OR B: 4x20g every 3h (INT) OR C: 2x40g every 6h (BOLUS)	All protocols \uparrow MPS above rest, INT elicited $>$ MPS than PULSE & BOLUS ($P < 0.05$) throughout the 12h recovery period.
Burd <i>et al.</i> (2011)	15 recreationally active men (21 ± 1 years) with previous RE experience.	Effect of exercise-mediated enhancement of MPS following 15g whey ingested at rest and 24 h after RE performed until failure of different exercise intensities (30% vs. 90%).	RE performed until failure following ingestion of 15g whey has a sensitizing effect on the myofibrillar prot fraction for at least 24 h following
Hoffman <i>et al.</i> (2009)	33 resistant-trained men (19.9 ± 1.3 years) Parallel design	Effect of 10-week protein-supplement timing trial on strenght and body composition A: Prot ingested morning & evening B: Prot ingested before and after workouts. C: Placebo	Strength improved in all 3 groups over a period of 10-weeks However, no significant differences were shown for strength or body composition between the 3 groups.
Cribb & Hayes, (2007)	23 recreational male bodybuilders Double-blind, randomized parallel design	Effect of 10-week protein-supplement timing trial on strenght, muscle mass and body composition A: Prot/ creatine/glucose (1g/kg/BM) ingested morning & evening (MOR-EVE) B: Prot/ creatine/glucose (1g/kg/BM) ingested before and after workouts (PRE-POST).	PRE-POST $>$ \uparrow in lean BM & 1RM ($P < 0.05$). PRE-POST also resulted in higher muscle creatine & glycogen values after the training program ($P < 0.05$).

RE= Resistance exercise; INT= Intermediate; MPS= Muscle Protein Synthesis; Prot = Protein; RM= Repetition maximum; BM= Body Mass;

A number of studies have examined the impact of protein ingestion during and after endurance exercise (van Loon *et al.*, 2000:106; Ivy *et al.*, 2004:136; Saunders *et al.*, 2007:678). Table 2.3 provides a summary of these studies that have specifically examined the effects of protein consumption during and after endurance exercise. Protein ingestion during endurance exercise has the potential to serve as fuel for oxidation, and can also stimulate cellular responses that can benefit exercise (Kreider *et al.*, 2010:9). Growing evidence now suggests that CHO and protein beverages improve endurance performance and post-exercise recovery. Saunders *et al.* (2007:678) have reported a significant improvement in endurance performance when athletes consumed carbohydrate in combination with protein versus carbohydrate-matched beverages (Table 2.3). Saunders *et al.* (2007:678) also suggested that the addition of protein to CHO during exercise resulted in reduced muscle damage as demonstrated by the increased creatine kinase concentrations following exercise in the CHO trial vs. the CHO plus protein trial (Table 2.3). In addition van Loon *et al.* (2000:106) and Ivy *et al.* (2004:136) have shown CHO plus protein beverages consumed during or immediately after exercise have been associated with improved muscle glycogen recovery (Table 2.3). Ivy & Portman, (2004:28) also reported that protein timing strategies can produce dramatic improvements in body composition, particularly with respect to increases in fat free mass.

2.3.3 Protein recommendations

The International Olympic Committee (IOC) protein recommendations for endurance athletes are 1.3 - 1.8 g/kg BW/day and 1.6 - 1.7 g/kg BW/day for strength-training athletes (Slater & Phillips, 2011:71). An even a higher intake (1.5 - 2.0g/kg body weight per day) for individuals taking part in intense or high volume training are recommended (Kreider *et al.*, 2010). The focus should be on eating high-quality protein throughout the day. The period after training is very important and it is suggested that a person consume 20 g to 25 g of high-quality protein 30-60 minutes after training to optimise MPS.

There appears to be a difference between the recommendations provided by the ACSM, ISSN and IOC. It is recommended that athletes who want to increase muscle mass and reduce body fat should follow IOC guidelines (Phillips & van Loon, 2011:34). The recommendations of the ISSN are based on training volume and intensity (Kreider *et al.*, 2010:9). The ISSN guidelines are based on publications by the same author and do not include the entire spectrum of published papers on protein intake and exercise. Guidelines for protein intake before and during exercise are provided by the ISSN only, while the ACSM advocates a “moderate” intake of protein before exercise (Potgieter, 2013:11).

Table 2.3: Effect of protein consumption during and after endurance training

Reference	Subjects	Protocol	Findings
Ivy <i>et al.</i> (2002)	7 trained male cyclists (23±1 years) Rank-order design	A: 80g CHO + 28g Prot + 6g fat (CHO-Prot) vs. B: 80g CHO + 6g fat (CHO) vs. C: 108g CHO + 6g fat (High CHO) immediately and 2h post muscle glycogen depletion exercise	After 240 min recovery, muscle glycogen significantly > for CHO-Pro vs. CHO and High CHO (88.8±4.4 vs. 70.0±2.8 vs. 75.5±2.8 mmol/l, respectively, P<0.05).
van Loon <i>et al.</i> (2000)	8 well-trained male cyclists or triathletes (24.0±0.6 years)	A: 0.8g CHO/kg/h (CHO) vs. B: 0.8g CHO/kg/h + 0.4 g wheat prot + leucine & phenylalanine (CHO-Prot) vs. C: 1.2 g CHO/kg/h (High CHO) received every 30-min during cycle to glycogen-depletion.	Muscle glycogen synthesis was higher in the CHO-Prot and High CHO trial vs. the CHO trial (35.4±5.1 and 44.8±6.8 vs. 16.6±7.8 µmol glycosol units/g dry weight, respectively, P<0.05). Addition of protein to a CHO solution or providing sufficient CHO every 30 minutes can accelerate glycogen synthesis.
Saunders <i>et al.</i> (2007)	13 recreationally competitive cyclists (8 men, 5 women) Randomly counterbalanced double-blind design.	Effect of consuming a gel containing A: 0.15 g CHO kg/BW (CHO) vs. B: 0.15 g CHO + 0.038 g protein kg/BW (CHO-Prot) every 15-minutes during timed cycle-trials to exhaustion	Exercise duration ↑ on CHO-Prot gel vs. CHO gel (116.6±28.5 vs. 102.8±25.0 min, p<0.05). CK ↑ significantly following the CHO trial vs. the CHO-Prot trial (183±116 vs. 267±214 U/L).
Burke <i>et al.</i> (2012)	2 crossover designed studies Study 1: 15 <u>untrained</u> male & female subjects (24±4 years) Study 2: 10 healthy endurance- <u>trained</u> men & women cyclists and triathletes (25±6 years)	Study 1: overnight-fasted subjects received 20 g prot from skim milk, soy milk, beefsteak, boiled egg, liquid meal supplement. Study 2: overnight-fasted subjects received 20 g prot from prot-rich sports bar at rest & after 60-min submaximal ride to characterize plasma amino acid response to the intake of food and exercise.	Amino acidemia patterns showed differences between foods. Liquid prot forms achieve peak concentrations 2x quicker after ingestion vs solid prot-rich foods (50 min vs 100 min). Skim milk achieved significantly faster peak leucine concentrations. Completing exercise before ingestion increased elimination rate (20–40%faster.)

CHO = Carbohydrates; Prot = Protein; CK = Creatine Kinase; kg = Kilogram

The sooner athletes consume protein after exercise the better, additionally frequent protein ingestion (every 3 - 4 h) over 24 h after exercise can sustain protein balance and fuel the elevation in MPS (Phillips *et al.*, 2012:160). Post-exercise ingestion (immediately to 3 hours post) of amino acids, primarily essential amino acids, has been shown to stimulate strong increases in muscle protein synthesis (Tipton *et al.*, 1999:628; Borsheim *et al.*, 2004:648). Daily post-exercise ingestion protein supplement promotes greater increases in strength and improvements in lean tissue and body fat percentage during regular resistance training.

2.3.3.1 Protein before exercise

The ACSM recommends adding moderate amounts of protein to a pre-event meal (Rodriquez *et al.*, 2009:515). The ISSN recommends, depending on exercise duration and fitness level, of the individual that protein should be included with carbohydrates in the pre-event meal before resistance exercise or when changes in body composition is required (Kerksick *et al.*, 2008:24). Consuming 0.15-0.25 g/kg BW protein with 1-2 g/kg BW carbohydrates in the pre-event meal 3-4 hours before training or competition is recommended (Kerksick *et al.*, 2008:24). Currently the IOC did not set any pre-exercise recommendations (Slater & Phillips, 2011:71).

2.3.3.2 Protein during exercise

ISSN concluded that protein added to CHO (CHO:protein ratio of 3-4:1) during exercise has shown potential in recent literature (Cermak, 2012:1454; Slater, 2011:71). The IOC refers to recent evidence that suggests co-ingestion of CHO and EAAs is beneficial before and during resistance exercise to increases substrate availability and exercise performance, improves anabolic hormonal environment, stimulates muscle protein synthesis and decreases muscle damage or tenderness (Slater & Phillips, 2011:71).

2.3.3.3 Protein after exercise

The ISSN recommends adding protein to CHO (CHO:protein ratio 3-4:1), or by supplementing with 0.2-0.5 g/kg BW protein for recovery. This results in increased glycogen resynthesizes and eventually improved performance (Kerksick *et al.*, 2008:25). They also recommends that 6-20g EAAs with 30-40 g high glycemic CHO, ingested immediately or within three hours after exercise stimulates muscle protein synthesis, increased strength and enhanced body composition during enduring resistance training (Kerksick *et al.*, 2008:25). The IOC opinion is that protein should be ingested after exercise at a time that is associated with optimal muscle protein synthesis (Slater & Phillips, 2011:71). They recommends 20-25 g of high quality/or high biological value protein after resistance exercise (Phillips & van Loon, 2011:32). The combination of carbohydrates and protein post exercise is important to restore muscle glycogen and promote protein synthesis (Slater & Phillips, 2011:71). Protein intake that exceeds this

recommended amount does not promote muscle protein synthesis, but can lead to protein oxidation. The dietary protein form of choice is flavoured low fat milk. It shows beneficial improvements in muscle synthesis (Phillips & van Loon, 2011:23). Table 6 is a summary of guidelines and recommendations set by the IOC, ACSM and ISSN.

Table 2.4: Recommendations from the IOC, ACSM and ISSN on the timing of protein ingestion.

Resistance exercise	Before	During	After	General
IOC	No recommendation	No recommendation	0.3 g/kg BW/h whey protein	1.3-1.8 g/kg BW
ACSM	Mod amount of prot added to pre-event meal 3-4 h before training.	BCAA during training	6-20 g EAA	1.2-1.7 g/kg BW
ISSN	No recommendation	CHO:PROT (3-4:1) BCCA	CHO:PROT (3-4:1)	No recommendations
Endurance exercise	Before	During	After	General
IOC	Pre-event meal: 0.15-0.25 g/kg BW prot Whey & egg proteins	EAA Protein/ CHO combination	20-25g high quality/or high biological value protein	1.6-1.7 g/kg BW
ACSM	Mod amount of prot added to pre-event meal	No recommendation	6g EAA + 35g sucrose	1.2-1.7 g/kg BW
ISSN	0.15 - 0.25g/kg BW	CHO:PROT (3-4:1)	6-20g high quality protein (EAA), soon after (30min-2h) exercise	No recommendations

IOC = International Olympic Committee; ACSM =American College of Sport Medicine ;

ISSN = International Society for Sport Nutrition; BCAA = Branched Chain Amino Acid;

CHO = Carbohydrates; Prot = Protein ; Mod = Moderate; BW = Body Weight

2.4 Importance of protein in rugby players

2.4.1 Description and physical demands of rugby

Rugby is widely considered as one of the most popular sports watched by millions of people throughout the world (Lako *et al.*, 2010:600). Rugby has become a multi-million dollar sport that places extreme physical demands on the players performing at the top and those aspiring to perform at the top. Rugby players compete for fame and glory in the rugby union or rugby league and to perform well, minimize mistakes and avoid injury rugby players have to be physically fit and strong, well-nourished, skilled, psychologically alert and determined (Duthie *et al.*, 2003:202)

Rugby is considered a team sport and a rugby team consists of 15 players, of which eight players are regarded as forwards and seven players are regarded as backs. The total duration of a rugby game is 80 minutes divided into two 40-minute halves with a 10-minute break between the two halves (Gabbette, 2005:1273). Rugby can be described as a high work rate, multi-activity contact sport with irregular, high intensity interval sessions (running and passing, sprinting and tackling) and longer periods of lower intensity activities such as walking, jogging and standing (Duthie *et al.*, 2003:202, Gabbette, 2005:1273, Roberts *et al.*, 2011:1253). High intensity efforts last between 5-15 seconds while the low-intensity or rest periods last up to 40 seconds. During a typical rugby game the total distance covered by players can be broken down into 34% sprinting, 28% jogging and 37% walking. Professional rugby players require high levels of endurance and stamina to play this very physical sport. The aerobic system provides up to 60% of energy required although most energy supplied during ball-in-play time will be provided by the anaerobic system (35-45%) in high level players. Rugby players therefore require considerable aerobic and anaerobic qualities, as well as muscular strength and power and speed (Duthie *et al.*, 2003:202). Rugby players have unique nutritional requirements and the appropriate timing of meals and supplements play an important role (Petrie *et al.*, 2004:620).

2.4.2 Importance of nutrition and specifically protein to rugby players

Optimal nutrition has been shown to improve and enhance sports performance (Roberts *et al.*, 2011:1253). Today, more than ever, athletes aim to maximize the practise of ingesting the right nutrients, in the correct amounts, at an appropriate time to improve performance. Although diet alone is not enough to directly increase strength, power and aerobic endurance, a sound diet will allow players to train optimally and compete to the best of their ability (Maughan & Gleeson, 2010:94). Good nutrition is essential for rugby players to meet the physical demands of training, and for achieving peak performance on match days. Proper nutrition could ensure adequate energy levels for lean mass development that is desirable in rugby players to enhance strength and power (Duthie *et al.*, 2003:202).

Due to the physical nature of the sport, it is not uncommon for rugby players to present with muscle damage as indicated by elevated systemic markers of muscle damage such as plasma CK and myoglobin (Roberts *et al.*, 2011:1253; Smart *et al.*, 2008:19). It is likely that this damage occurs not only as a result of physical impact, but also as a result of mechanical and metabolic stress associated with exercise (Lambert *et al.*, 2005:52; Smart *et al.*, 2008:8). The resulting pain and reduction in muscle function might be considered a limitation to training and match performance. To maintain physical and skill attributes throughout a season, it is important that interventions are identified that allow players to recover as quickly as possible (Roberts *et al.*, 2011:1253).

Rugby players are particularly involved in strength and power training to optimize muscle mass and strength for the physical side of the game, however endurance is also a component of the game and is therefore also included in the training program. Protein requirements increase for both endurance training as well as resistance training, therefore protein is an important consideration for rugby players. For the endurance component, underlying mechanisms include tissue repair and the use of the BCAA's for supplementary fuel (Wilson & Wilson, 2006:12). Whereas for the strength and power component, the mechanisms are tissue repair and the maintenance of positive nitrogen balance to maximize hypertrophy stimulus (Burd *et al.*, 2011:568). Considering that heavy resistance exercise results in damage to the active muscle fibers, a greater protein intake may assist in repairing these fibers (Tipton *et al.*, 2004:2073). Indeed a decrease in muscle damage and enhanced recovery from resistance exercise has been demonstrated in subjects using protein supplements (Ratamess *et al.*, 2003:637). The combination of resistance training with the addition of amino acids results in a positive nitrogen balance and an increase in protein synthesis (Rennie & Tipton, 2000:476; Wilkinson *et al.*, 2007:3704) potentially resulting in improvements in both muscle size and strength. Protein intake has also been suggested to have an important role in regulating the anabolic hormones involved in muscle remodeling (Rennie & Tipton, 2000:471). Ingestion of protein during or after exercise has been reported to reduce perceived muscle soreness and systemic markers of muscle damage (Betts *et al.*, 2009:773) and to accelerate post-exercise recovery of muscle function following prolonged exercise (Roberts *et al.*, 2011:1253).

2.4.3 Protein requirements for rugby players

Considering rugby a sport with aerobic, anaerobic and strength components, the following protein applications are applicable. It is important that rugby players consume adequate amounts of protein rich foods each day to promote adaptations and recovery from games (Lundy *et al.*, 2006:199). General recommendations for protein intake amongst rugby players are players are 1.5 - 2.0g per kg of body weight a day (Lundy *et al.*, 2006:209). Players who want to maintain or increase their muscle mass could consume between 1.8 - 2.0g per kg of body weight a day. This is considered a high intake and should only be undertaken if the player is regularly training at high intensities (Lundy *et al.*, 2006:210).

Animal based protein is superior to most plant based protein. Animal protein sources provide all EAAs considered as high-quality complete protein for instance egg, milk and whey (Kreider *et al.*, 2010:9; Hulmi *et al.*, 2010:51). Lean meat, chicken, fish and skim milk is also excellent sources of protein due to their high protein content and low fat content and are therefore advised to rugby players as protein sources. Rugby players should aim to include protein rich food throughout the day and specifically around training sessions (Lundy *et al.*, 2006:199). Low

fat flavoured milk is the best protein form to consume after exercise and has shown improvements in muscle synthesis (Potgieter, 2013:12)

The literature demonstrates that there is a limited time window within which to ingest protein for optimal synthesis (Witard, 2014:86; Aragon & Schoenfeld, 2013:7). Studies indicate that ingesting protein prior to, during or following intense exercise influence protein synthesis pathways (Tipton *et al.*, 2008:89; Willoughby *et al.*, 2007:476).

Researchers have examined the effects of types and timing of protein ingestion on various physical changes in weight trainers. An ideal protein after resistance exercise should contain whey protein providing 3-4 g of leucine as this will rapidly digest and promote maximal MPS (Hoffman *et al.*, 2009:173). Such a source would be ideal for rugby players to increasing muscle protein synthesis, resulting in increased muscle hypertrophy and strength.

In general, protein before or after training have shown to increase physical performance (Hoffman *et al.*, 2007:85; Arendt *et al.*, 2009:74), improve exercise recovery (Roberts *et al.*, 2011:1253), improve lean body mass (Arendt *et al.*, 2009:74), enhance muscle hypertrophy (Hulmi *et al.*, 2009:52) and strength (Hoffman *et al.*, 2007:86). Table 2.5 provides a summary of studies that have examined aspects of protein consumption specifically in university rugby players. Habitual protein intake was within or higher than the recommendations for athletes (Potgieter *et al.*, 2014:35, Alghannam *et al.* 2011:748). Pre- and post-game protein intake also met the requirements. Furthermore when protein was included during exercise, running capacity was enhanced (Alghannam *et al.* 2011:748)

Table 2.5: Summary of studies examining protein consumption during or around training in rugby players

Reference	Subjects	Methodology	Results
Potgieter <i>et al.</i> (2014)	35 Maties Varsity cup rugby players male (21.9±1.2years)	A questionnaire was completed to determine match-day dietary intake and habitual dietary intake (including protein).	Higher than recommended intakes for total protein (2.4 (0.7) g/kg BW) were consumed. Nutritional match-day strategies were excessive for protein (1.2 (0.6) g/kg BW) in the pre-event meal, also post-event meal consumed higher than recommended protein intakes (0.9(0.4) g/kg BW).
Lako <i>et al.</i> (2010)	19 elite Fiji 15-a-side rugby players 22-31 years	Attended a workshop where food questionnaires were administered. The questionnaire had 4 sections: personal detail, food frequency, typical preparation diet 7 days prior to match and on match days and 2 x 24h food diary.	Energy contribution from the 3 major macronutrients during the pre-match meal was CHO (49%), Prot (15%) and fat (36%) while post match meal CHO (44%), Prot (20%) and fat (36%). The study revealed poor eating habits of the players resulted in low CHO intake, which may contribute to increased levels of fatigue leading to stamina loss, even before the second halve of the game. It appears that the diet of most players does not provide enough energy.
Schokman <i>et al.</i> (1999)	N=40 male elite Australian Football players from Carlton football club	Dietary info collected via 4-day weighed-food records, 2 days before and 2days after competition. $\frac{2}{3}$ players recorded pre and postgame dietary intakes and remaining recorded post then pre.	Mean protein intake was 1.6g/kg BW for pre and post-game meals, respectively. This was higher than recommended intakes for pre and post protein intakes.

CHO= Carbohydrates; Prot= Protein; BF= Body Fat; FM= Fat Mass; TC= Total Cholesterol; LBM= Lean Body Mass.

2.4.4 Knowledge and perceptions of protein timing in university rugby players

Table 2.6 is a summary of studies previously done on nutritional knowledge of rugby or football players. Young rugby players in a study conducted by Walsh *et al.* (2012:365) demonstrated poor nutritional knowledge on how to maximize sport performance (mean knowledge score $59.7 \pm 12.8\%$). Of these senior schoolboy rugby players examined by Walsh *et al.* (2012:365), 61.6% followed the recommendations to refuel immediately after exercise, whilst the rest of participants only refueled more than 30 min after exercise. Before exercise high-carbohydrate foods were consumed by 88% of participants and high-protein foods were consumed by 94.4%. Participants in this study also demonstrated poor applied knowledge, with 62.1% (incorrectly) believing that steak and salad, which provides a negligible amount of carbohydrate, is a good refueling meal (Walsh *et al.*, 2012:365). Walsh *et al.* (2012:365) concluded that young rugby players have a poor understanding of the role of protein in sport (mean knowledge score of 39.2%). Moreover, despite being advised, frequently, on protein requirements for sport, this was the area in which most players had a deficit in nutritional knowledge, reinforcing the perception that the nutritional advice given may be inaccurate or inappropriate (Zinn *et al.*, 2006:214).

Strachan *et al.* (2009:2), assessed the knowledge of adolescent rugby-playing boys from Kwazulu-Natal, South Africa, regarding protein and creatine, two of the most commonly used supplements. Both creatine (88%) and protein (56%) were thought to increase strength. Neither were thought to increase endurance (88% and 81% for creatine and protein, respectively). Fifty percent of the players thought that creatine would enable one to train harder, compared to only 25% who thought protein facilitate harder training. Sixty three percent of the participants thought both creatine and protein resulted in muscle mass gain. The majority felt that neither creatine nor protein would increase energy levels (75% and 81%, respectively). Fifty percent of the participants thought that creatine aid recovery while 56% thought that protein aid recovery (Strachan *et al.*, 2009:2).

Table 2.6: Summary of studies examining nutrition knowledge in rugby players

Reference	Subjects	Methodology	Results
Alaunyte <i>et al.</i> (2015)	21 First team players (age 25±5) professional rugby league players England	Nutrition knowledge Q of 3 sections: A– recommendations & advice; B – food groups - classify food items into groups; C – food choice - choosing healthier food. Q distributed prior to a training session & collected after completion.	The average number of nutritional knowledge questions answered correctly was 52.4±4.4%, in this group of athletes with the highest scores in dietary advice section (85.7%), followed by food groups (71.2%) and food choice (69.5%).
Duvenage <i>et al.</i> (2015)	198 Elite u/16 male rugby players in South Africa	Completed a dietary questionnaire specifically designed for this study. The questionnaire was designed to investigate basic ideas and concepts around nutrition knowledge and supplement usage.	Players had better knowledge about nutrition recovery strategies than pre-game meals. 70% identified optimal timing and 56% knew the ideal macronutrient composition of recovery meals. Over 60% of players believed supplements were safe and necessary for increasing muscle mass, and almost half believed they could take creatine. Over a third also believed that the protein quality of supplements was higher than that of food. Elite U16 rugby players in this study lacked comprehensive sports nutritional knowledge
Walsh <i>et al.</i> (2011)	203 15 - 18 year male rugby players from Ireland	The initial knowledge questionnaire was piloted by 4 male secondary-school boys. Minor changes made to wording & order of the questionnaire which consisted of 40 questions in 5 sections: position of play, training, dietary intake, hydration practices, nutrition attitudes, nutrition knowledge and sources.	59.6% answered Q correctly. Q's on protein=most poorly (39.2%). 82.8% aware to eat immediately after training, 62.1% incorrectly ID steak & salad as a suitable meal before/ after exercise. 36% knew muscles do not get most E from prot. 39.4% knew excess prot consumed = stored as fat, 38.4% believed that the more prot you eat the more muscle you build. The group highlighted disparity between knowledge & practices. 68 athletes knew they should eat immediately after exercise. After a match or training session, 61.6% ate within ½ an hour. Before exercise, 96.1% consumed high-CHO foods. High-prot foods were consumed by 80.8% of players before exercise. After exercise, 85.7% consumed high-CHO foods & 92.1% high-prot foods.

Table 2.6: Summary of studies examining nutrition knowledge in rugby players (continue)

Reference	Subjects	Methodology	Results
Burkhardt, (2010)	100 participants from which 28 is rugby players age 19±1.1 from a national sporting organization in New Zealand	Q addressed 4 aims. Section A- assess basic nutritional knowledge. Section B- investigate influences on food choice and food availability in the subjects. Section C - assess the basic sports nutrition knowledge and practices of the subjects and section D basic demographic information about the subjects.	Rugby players in particular score 0% on questions regarding pre-event food, 17.84% answered correctly on questions about respectively fluid and food consumption during an event, while 14.29% were correct about the amount of recovery food to consume. Surprisingly 71.43% answered questions correctly regarding the time to consume recovery food.
Strachan, (2009)	68 male rugby players (16 - 18years) from 7 KZN Schools in South Africa	The questionnaire investigated perception and usage practices among schoolboy rugby players.	54% use nutrition supplements. Protein & creatine was mostly used (43% & 22%). Peers are seen as good source of info. Peer pressure lead to misuse of supplements & detrimental side-effects. School-level education program improve supplement usage practices & creating sound nut practices and making informed decisions.
Alford <i>et al.</i> (2004)	21 pro and 24 semi-pro football players (18 - 35 years)	The Q comprised of 4 sections: 1. Habits (eating & drinking habits before, during, post comp & training). 2. Nutritional knowledge (on pre-match meals, CHO, fat, protein, fluids, supplements, training, weight control). 3. Believes/personal info.	A difference was found in between pro and semi-pro rugby players in the type of meal consumed pre-match. Pro players reported eating food high in CHO and protein, while semi-pro only consumed high CHO food. Just 13% (pro and semi-pro) players consumed food during half time (CHO rich) with no significant difference between groups. Most players ate food within 1.1 hour after completion of the match.

Q = Questionnaire; CHO = Carbohydrates; Pro = Protein; KZN = Kwazulu Natal

Table 2.7 is a summary of studies previously done on nutritional knowledge of collegiate football players. In a study conducted by Alford *et al.* (2004:1) on “Nutritional Knowledge and Dietary Habits of Professional and Semi-Professional Football Players”, good knowledge was shown when considering the type of pre-meals, and the recommended timing of meals and snacks. All the professional football players and the majority of semi-professional players correctly identified that pre-match meals should be consumed 3-4 hours before the match. Sixty seven percent of all football players correctly identified that snacks should be consumed 2 hours before kickoff. When looking at the types of food to consume before a match, pasta was correctly identified as a good meal, as was beans on toast (Alford *et al.*, 2004:2).

Various studies supported the lack of knowledge in protein among college athletes (Rash *et al.*, 2008, Torres-McGehee *et al.*, 2012:205, Fox *et al.*, 2011:9). In a recent studies, Torres-McGehee *et al.* (2012:205), revealed a lack of nutritional knowledge (54.9%±13.5 mean knowledge score) among 185 university athletes from diverse sport codes including football. Knowledge was poor for all domains (macro- and micronutrients, supplements related to performance, weight management and associated eating disorders, as well as hydration). The section on macro- and micronutrients (including protein) received the lowest mean knowledge score for all participants. Hale, (2013) also noticed a lack of knowledge in freshman football players with regards to protein, these football players believe by increasing protein levels, muscle size will also increase. Seventy five percent of these participants believed an increased protein consumption will contribute to increased muscle size while 13% of the participants believed that protein alone can lead to increased muscle size. Football teams traditionally focus on weight gain through strength training and supplements such as protein shakes and bars, but simple daily dietary habits are left untouched (Hale, 2013:2).

While some studies have been performed on collegiate athletes and to a lesser extent on collegiate football players to determine knowledge on nutrition and protein, none has been done specifically on collegiate football players’ knowledge on the timing of protein intake, none the less in South African Universities on rugby players.

Table 2.7: Nutrition knowledge and perceptions of college athletes including rugby/football players

Reference	Subjects	Methodology	Results
Hale, (2013)	45 freshman (18-26yr) senior football players in Utah	A questionnaire was used as a tool for determining nutrition habits of college football athletes. The % of athletes answering each question in each category correctly was calculated.	86% athletes were unaware that nutritionists are available. 60% was interested in meeting a nutritionist. 46% rely on strength coaches for guidance & 25.5% on teammates/friends & family. Research on dietary interventions is helpful to determine ways to improve knowledge on individual & team basis.
Torres-McGehee <i>et al.</i> (2012)	185 collegiate athletes in the USA	Sports nutrition knowledge (20 multiple-choice Q's). 4 domains: micro & macro nutrients, supplements & performance, weight management, eating disorders & hydration. A score of 75% or more = adequate nutrition knowledge, ≤ 75%= inadequate nutrition knowledge. Level of confidence measured by 4-point Likert scale (1 =not at all, 2=not very, 3=somewhat, 4 = very confident).	68.5% in all domains. Adequate knowledge in 35.9% coaches, 71.4% ATs, 83.1 % SCSs, & 9% athletes. Most used nutrition resources for coaches, ATs & SCSs = RD's. ATs & SCSs have adequate sports nutrition knowledge, coaches & athletes have inadequate knowledge. Athletes have frequent contact with ATs & SCSs; therefore, proper nutrition education among them is critical. Proper nutrition programs should be provided for athletes, coaches, ATs, & SCSs.
Rosenbloom <i>et al.</i> (2002)	N = 328, males =237; females = 91 collegiate athletes in Georgia	The nutrition questionnaire used for study was developed by the author.	Collegiate athletes recognize the importance of CHO and fluid in performance. The role of protein seems to be unclear to these collegiate athletes. Education on protein as a fuel and its role in building muscle.

Q= Questionnaire; AT= Athletic Trainers; SCS= Strength and Conditioning Specialists; RD= Registered Dieticians; CHO= Carbohydrates'

CHAPTER 3: ARTICLE 1

The article entitled: “**Development, validation and reliability of a questionnaire to determine the knowledge of athletes on the timing of protein ingestion**” was submitted to the Journal of Nutrition Education and Behaviour. The article is therefore written according to the author instructions for the Journal of Nutrition Education and Behaviour (see Annexure F) with the exception of placing the tables and figures in text and not in separate pages at the end as per author instructions.

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Running head: Development and validation of a knowledge questionnaire on timing of protein ingestion

ABSTRACT

Objective: Development and validation of a reliable questionnaire assessing athlete's knowledge on timing of protein ingestion.

Design: Observational, test-retest study.

Setting: North–West University, South-Africa.

Participants: 70 university athletes.

Methods: The questionnaire was developed through in-depth literature reviews, evaluated for content validity and tested for face validity. The questionnaire was administered to the same group 15 days apart to test construct validity and reliability.

Main outcomes: Validity and reliability.

Analysis: Item difficulty index, inter-item correlation (Cronbach's alpha (CA)) and item discrimination (CA) determined construct validity. CA, *t* test, mean percentage difference, intra-class correlation (ICC), Kappa and Bland-Altman assessed reliability.

Results: A 12-item questionnaire was developed. Item difficulty of 11 questions was good (range 8.6% - 84.3%), strength of consistency was good (CA = 0.31), but internal consistency was poor (CA = 0.31). The questionnaire is reliable at group level for agreement (*t* test $P = .078$) and association (Bland-Altman indicated 95.7% within limits of agreement). On individual level the questionnaire showed limited bias ($P = .072$), limited error (CA = 0.64), limited agreement (Kappa = 0.13), but strong association (ICC = 0.64).

Conclusion and implications: The questionnaire is relevant, clear and valid and provides information on knowledge on protein timing.

INTRODUCTION

Nutrition and performance are topics of importance to serious athletes.¹ Training and competition can be intense and optimal nutrition can separate winning from losing.^{1,2} Correct timing of protein intake for instance has been identified as an important component to optimize the adaptive response to both resistance and endurance exercise training.³ Research conducted by Cermak, et al.⁴ and McLain, et al.⁵ indicated that timely consumption of protein before, during and after exercise has the ability to increase muscle protein synthesis, muscle glycogen restoration, muscle damage repair, muscle size, muscle strength and potentially performance.^{4,5,6} Previous research indicated that timely consumption of protein before, during and after exercise has the ability to increase muscle protein synthesis, muscle glycogen restoration, muscle damage repair, muscle size, muscle strength and potentially performance.^{4,5,6}

Nutrition knowledge influences what and when athletes eat^{7,8} and can therefore influence performance. Limited research has however been conducted on nutritional knowledge of athletes, specifically regarding timing of protein intake. Accurate knowledge assessment firstly requires a valid and reliable questionnaire.⁹ A valid questionnaire asks the right questions and provides a reflection of the truth.¹⁰ A reliable questionnaire further provides similar information when administered at multiple occasions and is therefore consistent.¹¹ Both these qualities are essential for a sound knowledge questionnaire.

Formulating a framework from the literature on which to base the content of the questionnaire is the first step in developing a valid and reliable questionnaire.¹⁰ Relevant questions are formulated and evaluated by an expert panel for content validity. Content validity refers to a question being appropriate and relevant to the study purpose and area of investigation.¹¹ The questionnaire is also tested for face validity to ensure questions are clear and correctly understood.¹² Construct validity determines how the predetermined content fit together and includes item-difficulty (questions should be on the same difficulty level), internal consistency (questions should conceptually fit together) and item discrimination (questions should be relevant to each other). Various statistical tests exist to assess different aspects of validity and reliability of questionnaires.¹⁰ For a comprehensive evaluation of a questionnaire, all these aspects should be assessed. This includes the size and direction of error and agreement, association and bias for individual versus group assessments.¹⁰

A valid and reliable questionnaire assessing knowledge on timing of protein intake does not exist. The aim of this study was to develop and validate a reliable questionnaire regarding knowledge on timing of protein intake.

METHODS

This study was an observational study with a test-retest study component. Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Health Sciences, North-West University (NWU) (NWU-00022-15-S1), South Africa. Written informed consent was obtained before participation. The study was conducted in 3 phases: i) development, ii) validation iii) and reliability testing.¹¹ Data was captured in Microsoft® Excel and analysed with SPSS version 22. Normality was tested with Shapiro Wilk's test and non-parametric data were log transformed to improve distribution and interpretation of emerging patterns. Tables 1 and 2 present a summary of assessments and/or statistical tests and interpretations used to evaluate the questionnaire regarding content, face and construct validity (including item difficulty, internal consistency and item discrimination) and reliability.

PHASE 1: QUESTIONNAIRE DEVELOPMENT

Developing the questionnaire involved a thorough literature review for position papers and guidelines from the American College of Sport Medicine, International Olympic Committee and International Society of Sports Nutrition. Two databases were extensively reviewed (PubMed and Scopus) and a summary of the most recent and relevant guidelines on protein intake (before, during and after exercise) in strength and endurance athletes was compiled. Draft questions were formulated based on these.

PHASE 2: VALIDITY

Secondly, the questionnaire was tested for content, face and construct validity.

Content validity. The first draft questionnaire was sent to four experts in sports nutrition. They evaluated accuracy, representation and appropriateness of the knowledge questions on timing of protein intake.¹⁴ Feedback was incorporated and the questionnaire adjusted.

Face validity. The adjusted questionnaire was sent to five nutrition lecturers and five dietetic students at NWU for feedback on clarity of wording and layout. Feedback was incorporated and the questionnaire adjusted.

Construct validity. The final 12-item questionnaire was tested among 70 male NWU hockey and cricket players who volunteered participation. Athletes were included if they were a NWU hockey or cricket player enrolled for any degree excluding nutrition subject/s.

Verbal and written informed consent was obtained. The questionnaire was administered at their place of practice (Assessment 1). Construct validity was determined for item difficulty, internal consistency and item discrimination using data from assessment 1.

- *Item difficult index.* The total for each correctly answered question was determined and converted to a percentage ($x/70 \times 100$) and evaluated against the item difficulty index. The index classified questions as 'too easy' or 'too difficult' (poor).
- *Internal consistency.* Using the Cronbach's alpha, a total score for each question was computed to estimate the consistency of each individual question.
- *Item discrimination.* The Cronbach's alpha value for the entire questionnaire was calculated and recalculated after each question was individually omitted (11 questions) to specify if internal consistency increase with deletion of questions.¹⁰

PHASE 3: RELIABILITY

The test–retest method was used where the same questionnaire was tested on two separate occasions. The questionnaire was completed by the same participants 15 days later (Assessment 2). Various statistical tests were conducted to investigate different aspects of reliability including Cronbach's alpha, *t* test, percentage difference, correlation coefficients, Kappa statistics and Bland-Altman analysis (Table 2).

Cronbach's alpha. Results of the total score for Assessment 1 and 2 were used to determine internal consistency between the assessments. A high Cronbach's alpha value does not always indicate a high degree of internal consistency, because Cronbach's alpha is affected by the length of questionnaires. Thus a short questionnaire will by default result in a lower Cronbach's alpha value which is indicative of a high degree of internal consistency (Table 2).

t test. To determine agreement between Assessments 1 and 2 the two tailed *t* test was conducted to compare the total scores obtained for Assessment 1 and 2 (Table 2).

Mean percentage difference. The total score obtained for each question was used in the following formula: $[(\text{Assessment 1} - \text{Assessment 2}) / \text{Assessment 2}] \times 100$.¹⁸ The higher the mean percentage difference the larger the difference between the two answers. The same formula was applied to determine total score mean percentage difference for each assessment.

Intra-class correlation coefficient. A total percentage score was calculated for each assessment of the questionnaire (Assessment 1 and 2) and intraclass correlation coefficient (ICC) was calculated to determine inter-rater reliability (Table 2).

Kappa statistics. Total scores were classified into three knowledge groups: i) weak ($\leq 54\%$), ii) moderate (55% - 69%) and iii) good ($\geq 70\%$). The number '1' was allocated to athletes with a weak knowledge, '2' for those with moderate and '3' for those with good knowledge.²⁰ Weighted Kappa statistics were calculated for the total score of each participant. Athletes who achieved a score in the same category for Assessment 1 and 2 was also allocated a '1', those with scores in adjacent groups received a '0.5' and those with scores in opposite groups a '0'. Kappa statistics was also calculated for the total score on Assessment 1 and 2 (Table 2).

Bland-Altman analyses. Bland-Altman analyses included plotting the total score difference between the assessments (Assessment 1 – Assessment 2) (y-axis) against the mean of total score of the two assessments $[(\text{Assessment 1} - \text{Assessment 2}) / 2]$ (x-axis) for each athlete.²¹ The limits of agreement (LOA) were calculated as the mean difference ± 1.96 standard deviation (SD) and reflects over- or underestimation¹⁰ (Table 2). Pearson correlation coefficient (r) was calculated between the mean and the mean difference of the two assessments¹⁰ (Table 2).

TABLE 1: SUMMARY OF ASSESSMENT OR STATISTICAL TESTS USED TO EVALUATE VALIDITY OF QUESTIONNAIRE

Type of validity		Explanation	Aspect of validity	Assessment / Statistical test	Interpretation criteria	
					Good	Poor
Content validity		Questions are appropriate and relevant for study purpose and area of investigation ¹⁴	Relevance ¹⁴	Panel of 4 experts in the field of sports nutrition reviewed questions	N/A	N/A
Face validity		Clarity and logic layout of questionnaire ¹¹	Clarity ¹¹	Administration of questionnaire to 5 students and 5 lecturers	N/A	N/A
Construct	Item difficulty	Difficulty of individual questions	Difficulty ¹⁵	Item difficulty index ¹⁵	< 90% or > 10% of participants answer correct ¹⁵	> 90% or < 10% of participants answer correct ¹⁵
	Internal consistency	Extent to which individual questions fit together conceptually ¹⁰	Inter-item correlation ¹⁰	Cronbach's alpha ¹⁷	$\geq 0.7^{12,24}$	$< 0.7^{12,24}$
	Item discrimination	Extent to which single questions influence construct of the questionnaire ²⁴	Strength of consistency	Cronbach's alpha ^{17,24}	$\geq 0.2^{24}$	$< 0.2^{24}$

TABLE 2: SUMMARY OF STATISTICAL TESTS USED TO EVALUATE RELIABILITY OF QUESTIONNAIRE

Statistical test		Purpose	Aspect of reliability	Interpretation criteria		
				Good	Acceptable	Poor
Cronbach's alpha		Provides internal consistency. ¹⁰	Amount and error at individual level ¹⁰	0.9 – 0.95 ^{24,17}	> 0.7 ²⁴	<0.7
t test		Test agreement between two assessments of the same questionnaire. ¹⁰	Agreement at group level ¹⁰	P > .05 ^{19,26}		P ≤ .05 ^{19,26}
Percentage difference		Difference between two assessments to determine how close results are related. ¹⁰	Size and direction of agreement at group level ¹⁰		0.0 – 10.0% ^{19,26}	
Intraclass correlation coefficient		A measure of degree to which two assessments are associated. ¹⁰	Strength and direction of association at individual level ¹⁰	≥ 0.50 ^{24,26}	0.20 – 0.49 ^{24,26}	< 0.20 ^{24,26}
Kappa coefficient		Rank data into categories or groups and exclude chance agreement. Does not take into account size of disagreement between questionnaires. All disagreement are treated equally as total disagreement. ¹⁰	Agreement (excluding chance) at individual level ¹⁰	≥ 0.61 ^{10,26}	0.20 – 0.60 ^{10,26}	< 0.20 ^{10,26}
Bland Altman	Pearson correlation coefficient	Correlation between mean and mean difference of two assessments. ¹⁰ Reflects presence and direction of proportional bias. ¹⁰	Association between size and error.	P > .05 ^{24,26}		P ≤ .05 ^{24,26}
	LOA	Quantify agreement between two quantitative assessments which calculates, by using mean and standard deviations of differences between two assessments. ¹⁰	This quantifies the bias and a range of agreement ¹⁰	≥ 95% of N ^{24,26}		< 95% of N ^{24,26}
	Width LOA	Difference between upper and lower LOA and indicates the span between LOA. ¹⁰	Strength of agreement ¹⁰	< 50% difference ^{24,26}		> 50% difference ^{24,26}

LOA = Limits of agreement

RESULTS

PHASE 1: DEVELOPMENT

Initially a 19-item self-administered questionnaire was developed from relevant literature. Questions were formulated in true / false format or four option multiple choice questions.

PHASE 2: VALIDATION

Content validity. Based on comments from the expert reviewers the format of the questions was changed to five-option multiple choice questions to decrease the chance of accidental correct answers. The questionnaire was also shortened to 12 questions. The questions that were removed were either not directly related to the timing of protein intake or were not based on official timing of protein intake guidelines. Practical examples of foods with specific portion sizes were also included in a number of questions (i.e. 120 – 150g [palm size meat]).

Face validity. Grammar on one question was corrected, one question was rephrased and the line spacing between options was increased for easy completion.

TABLE 3: DESCRIPTION OF EACH QUESTION ON THE QUESTIONNAIRE

Question	Topic	Question
1	Definition	In a sport nutrition context, which one of the following statements explains 'the timing of protein intake' the best?
2	Athletes whom will benefit	Which of the following athletes can benefit from optimal timing of protein ingestion?
3	Performance benefit	Which one of the following is not a potential benefit of eating protein at the right time in context to your training session:
4	Amount before exercise	The recommended amount of protein to consume <i>before resistance</i> exercise in combination with carbohydrate for an 80kg athlete is:
5	Benefit before exercise	Protein ingested <i>before resistance</i> training can potentially:
6	Amount during exercise	What is the recommended ratio of carbohydrate-to-protein to consume <i>during</i> exercise?
7	Benefit during exercise	Protein consumption <i>during</i> exercise can potentially:
8	Role during resistance exercise	The role of protein consumed <i>during resistance</i> exercise is primarily:
9	Amount after exercise	The recommended amount of protein to consume <i>after resistance</i> exercise is:
10	Best time after exercise	The best time to consume protein <i>after</i> exercise or an event is?
11	Sources after exercise	Which one of the following protein sources has the greatest effect on muscle protein synthesis when consumed <i>after resistance</i> exercise?
12	Combination after exercise	Protein consumption in combination with carbohydrates <i>after</i> exercise is important to:

Construct validity.

- *Item difficulty.* Figure 1 represents the percentage athletes that answered each of the 12 questions for Assessment 1 correctly. Scores for the individual questions ranged from 8.6 % (Question 4) to 84.3% (Question 10). None of the questions were answered correctly by > 90% athletes and only question 4 was answered correctly by < 10% (therefore ‘too difficult’ and classified as poor). The item difficulty for the majority of questions was therefore good.

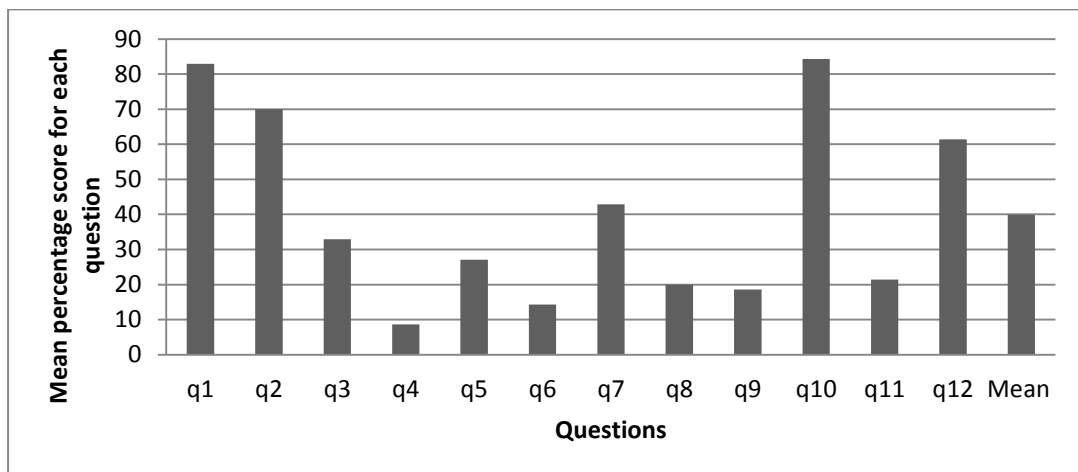


FIGURE 1: PERCENTAGE ATHLETES ANSWERING EACH QUESTION CORRECTLY

- *Internal consistency.* Internal consistency for Assessment 1 was poor with a Cronbach’s alpha value of 0.31 for the 12 questions.^{12,24}
- *Item discrimination.* Table 3 indicates the projected Cronbach’s alpha if individual questions were deleted from Assessment 1. Deleting questions 4, 10 and 11 will result in a higher Cronbach’s alpha (0.32, 0.34 and 0.39 respectively) (Table 4).

TABLE 4: ITEM DISCRIMINATION OF INDIVIDUAL QUESTIONS FOR ASSESSMENT 1

Cronbach alpha if item (question) deleted											
Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12
0.31	0.25	0.30	0.32	0.28	0.31	0.17	0.25	0.30	0.34	0.39	0.27

PHASE 3: RELIABILITY

Table 5 represents a summary of the main results from tests performed to test reliability of the knowledge questionnaire.

The Cronbach's alpha reliability test between Assessment 1 and 2 was 0.64, indicating a certain but acceptable amount of error at individual level (Table 5). A two tailed *t* test was conducted ($P = .078$) and indicated good agreement at group level between Assessment 1 and 2. The mean percentage difference between Assessment 1 and 2 ranged from -14.0 – 63.2%. Questions 1, 3, 5, 8 and 9 each had an unacceptable high percentage difference ($> 20.0\%$) while questions 2, 4, 6, 7, 10 and 11 were acceptable or good. Poor agreement at group and individual level was determined by mean percentage difference between total knowledge score on Assessment 1 and 2 of 24.1% and weighted Kappa statistics of (0.13), ranging from 0.15 - 0.48 indicating 8 questions had acceptable agreement at individual level. The ICC was determined as good association at individual level ($\alpha = 0.64$) with a significant value of ($P = < .50$) (Table 5). The LOA ranged from -25.8% - 32.2%, thus width of agreement was calculated as 58.0% indicating limited agreement (Table 5) although percentage agreement was determined as 95.7% ($\geq 95\%$ of participants within the LOA's). Pearson correlation coefficient between the mean and mean difference of total score for Assessment 1 and 2 was significant.

TABLE 5: RESULTS OF THE TESTS PERFORMED TO TEST RELIABILITY OF KNOWLEDGE QUESTIONNAIRE

	Cronbach's alpha	t test	Percentage difference	Interclass correlation	Kappa Statistics	Bland and Altman				
	α		(%)	r		Percentage Agreement	Lower limit	Upper limit	Width of LOA	Pearson correlation coefficient
Level of reliability	Individual	Group	Group	Individual	Individual	Group	Group		Group	Group
Cut off values	≥ 0.70	P > .05	0.0 - 10%	≥ 0.50	≥ 0.61	≥ 95% of N			< 50%	P > .05
Results	0.64	P = .078	24.1	0.64	0.13	95.7	-25.8	32.2	58.0	.072
Interpretation	Acceptable	Good	Poor	Good	Poor	Good	Good		Poor	Good

DISCUSSION

A valid and reliable questionnaire was developed to measure knowledge of athletes on the timing of protein intake. Very few studies involving the development and validation of tools to assess nutrition knowledge of athletes has been done, thus comparing results from this study against other relevant studies are difficult. However, the newly developed questionnaire can be used in future studies with the purpose to determine the knowledge of athletes regarding the timing of protein intake.

Rigorous development methods was used to develop the questionnaire^{11,15,24} and it complies with criteria for content, face and construct validity and thus for relevance, clarity, question difficulty, inter-item correlation and strength of consistency. Relevance and clarity was achieved by feedback from experts as well as lecturers and students in the nutrition department. Construct validity was further determined with the item difficulty index (difficulty level), internal consistence (inter-item correlation) and item discrimination (strength of consistency). The item difficulty index indicated that one question (Question 4) was considered too difficult, however this question was not removed because it was considered an important question when testing knowledge on the timing of protein intake.

Acceptable internal consistency is indicated with a minimum Cronbach's alpha = 0.6 for exploratory research.¹⁹ The low Cronbach's alpha results found in this study indicates poor linear relationship between the questions. In another study Whati et al,¹¹ developed a reliable and valid nutritional knowledge questionnaire for urban South African adolescents. They also reported a low internal consistency ($\alpha = 0.54$) for grade eight learners and children at disadvantaged schools ($\alpha = 0.52$) compared with multiracial groups at a multiracial school ($\alpha = 0.79$).¹¹ However, a high internal consistency ($\alpha = 0.77$) for all groups was reported. This could be due to the length of questionnaire (60 questions) and large sample size (N = 369). The low internal consistency reported in this study ($\alpha = 0.31$) could therefore be a result of the small sample size (N = 70) and short questionnaire (12 questions).²⁵

However, even with limited inter-item correlation, the questionnaire has been rigorously developed for content and relevance. Questionnaire length was therefore not increased merely to obtain a higher inter-item correlation.

Item discrimination determined if the questionnaire's consistency would be strengthened by removing specific questions. Deletion of 3 questions (Question 4, 10, 11) indicated a higher overall consistency of Assessment 1. These items were however retained because of content validity and relevance. Parameter and Wardle²⁴ concluded that an item-to-total-score correlation of 0.2 can be used as the cut-off value below which questions should be discarded.^{12,25} This was adhered to except in circumstances where an item was considered particularly important in terms of content validity.¹² The same was done on this study and thus no questions were removed.

In terms of reliability, the questionnaire successfully measured size and direction of agreement, association and bias for individual and group assessments. The questionnaire has a satisfactory internal consistency, although some questions lacked consistency, but were retained for improved content validity. Turconi et al,²⁵ used Pearson correlation coefficients for each section of their questionnaire and reported values ranging from 0.55 - 0.75 which agrees with results from the current study. Thus the amount and error at individual level was considered good.

No significant difference was found between Assessment 1 and 2 when the two tailed *t* test was conducted. This indicates good agreement between the two assessments at group level.

The mean percentage difference between total score obtained in Assessment 1 and 2 were high, indicating low agreement at group level. This could be because participants showed interest in the topic and discussed it with each other or did research during the 15 day period between assessments. Their resources were not necessarily scientific and thus influenced the results of the second assessment negatively. Malinauskas et al. 2007 concluded in a study among college athletes, aiming to investigate the sources of supplement information that internet (79.0%) was one of the most popular sources of supplement knowledge which is not per say the most reliable source²⁸.

Interclass correlation coefficient further indicated a positive relationship with a strong association between Assessments 1 and 2. This is an indication of good strength of association at individual level. Intraclass correlation was also used to assess test-retest reliability in a study conducted by Turconi.²⁵ Their coefficients ranged from 0.78 to 0.88, on the scores of the 68 students who completed the questionnaire twice, indicating a very good temporal stability of the questionnaire.

On the other hand, Kappa correlation coefficient indicated agreement of “no more than pure chance” between Assessments 1 and 2. The Kappa coefficient does not take into account the degree of disagreement. De Phino et al,²⁷ developed and validated a questionnaire to test the knowledge of primary care personnel regarding nutrition in obese adolescents. However, this study population were not familiar with the topic whereas De Phino et al,²⁷ used a population familiar with their research topic (personal care personnel).

Lastly, to illustrate the magnitude of agreement, identify outliers and trends in bias Bland-Altman analyses was done. Group agreement between the two assessments indicated that $\geq 95\%$ of the participants fell within the LOA and thus indicate good group agreement. The Pearson correlation was used to establish the association between size and error (or difference between two assessments) and the mean of two assessments. Data further indicated the lack of bias.

CONCLUSION

It is concluded that the newly developed questionnaire is relevant and clear. It is valid with a good difficulty level for the population. Although inter-item correlation is poor, strength of consistency is good. At group level the questionnaire is reliable, especially for agreement and association with no bias. In terms of reliability for individual use the questionnaire is reliable with little error and high strength of association. However agreement at individual level is limited and thus results relating to this should be interpreted with caution.

STRENGTHS AND LIMITATIONS

The strengths of the validation study included the fact that the validation study was conducted in a population (i.e. male university hockey and cricket players) that closely represents the population of the cross sectional study (i.e. male university rugby players). Only recruiting athletes specifically from North-West University are a limitation since this sample may not be representative of the general university athlete population. Future research should include student rugby players from other universities to compare knowledge in athletes at different universities. A stronger technique could have been used to determine content validity of the questionnaire, therefore future studies could make use of techniques such as Delphi. Future studies could also add questions around practices and supplement use of athletes to make this a more comprehensive questionnaire.

IMPLICATIONS FOR RESEARCH AND PRACTICE

The importance of protein timing in relation to sport is currently a highly debated area and more information on its effect on sport and physical activity outcome can improve athletes' performance and result in better health outcomes for those interested.

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REFERENCES

1. Torres-McGehee TM, Pritchett KL, Zippel D, et al. Sports nutrition knowledge among collegiate athletes, coaches, athletic trainers, and strength and conditioning specialists. *J Athl Train*, 2012;47:205–211.
2. Hale M. Nutritional Habits and Knowledge in the Division I collegiate football player. *All Graduate Plan B and other Reports Paper*, 283.
3. Phillips SM, Van Loon LJ. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci*, 2011;29:29-38.
4. Cermak NM, de Groot LC, Saris WH, et al. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr*, 2012;96:1454-1464.
5. McLain TA, Escobar KA, Kerksick CM. Protein application in sports nutrition - part 1: Requirement, quality, source, and option dose. *Strength Cond J*, 2015;37:61-71.
6. Hoffman JR, Ratamess NA, Kang J, et al. Effects of protein supplementation on muscular performance and resting hormonal changes in college football players. *J. Sport Sc. Med*, 2007;6, 85-92
7. Walsh M, Cartwright L, Corish C, et al. The body composition, nutritional knowledge, attitudes, behaviours, and future education needs of senior schoolboy rugby players in Ireland. *Int. J. Sport Nutr. Exerc. Metab.* 2011;21:365-376.
8. Barzegari A, Ebrahimi M, Azizi M, et al. A study of nutrition knowledge, attitudes and food habits of college students. *World Appl Sci J*. 2011;15:1012-1017.
9. Byrd-Bredbenner CA. A nutrition knowledge test for nutrition educators. *J Nut Educ*. 1981;13:97-99.
10. Lombard MJ, Steyn NP, Charlton KE, et al. Application and interpretation of multiple statistical tests to evaluate validity of dietary intake assessment methods. *Nutr J*. 2015;14 (1), 40.
11. Whati LH, Senekal M, Steyn NP, et al. Development of a reliable and valid knowledge questionnaire for urban South African adolescents. *Nutr*. 2005;21:76-85.

12. Kline P. *The Handbook of Psychological Testing*. Uitgawe. London, Routledge; 1993.
13. DeVon HA, Block ME, Moyle-Wright P, et al. A psychometric toolbox for testing validity and reliability. *J Nurs Scholarsh*. 2007;39:155-164.
14. Catsicas ME, Lombard M, Potgieter S, et al. (2014). An Assessment of the level of knowledge of health professionals on nutrition and diabetes self-management in treating patients with Type I and Type II Diabetes Mellitus in South Africa. *Cape Town: US*.
15. Whadi-ah T, Steyn NP, Visser M, et al. Development and validation of knowledge test for health professionals regarding lifestyle modification. *Nutr*. 2003;19:760-766.
16. Dwyer JT, Stoulorow KA, Orr R. Nutrition knowledge test for high school students. *J Nutr Educ*. 1981;13:93-94.
17. Tavakol M, Dennick R. Making sense of Cronbach's alpha. *Int J Med Educ*. 2011;2:53-55
18. Oduro-Kwarteng S. (2011). Private Sector Involvement in Urban Solid Waste Collection. *Leiden. CRC Press*.
19. Gibson R. *Principles of nutritional assessment*. 2nd ed. Oxford: Oxford University Press; 2005.
20. Sedek R, Tan YY. Dietary habits and nutrition knowledge among athletes and non-athletes in National University of Malaysia (UKM). *Pak. J. Nutr*. 2014;13:752-759.
21. Taren D, Dwyer J, Freedman L, et al. Dietary assessments methods: where do we go from here? *Public Health Nutr*. 2002;5:1001-3.
22. Haas M. Statistical methodology for reliability studies. *J Manipulative Physiol Ther*. 1991;14(2):119-32.
23. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Method Med Res*. 1999;8:135-60.
24. Parmenter K, Wardle J. Development of a general nutrition knowledge questionnaire for adults. *Eur J of Clin Nutr*. 1999;53:298-308.

25. Turconi G, Celsa M, Rezzani C, et al. Reliability of a dietary questionnaire on food habits, eating behaviour and nutritional knowledge of adolescents. *Eur J of Clin Nutr.* 2003;57:753-763.
26. Masson L, McNeill G, Tomany J, et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficient and the Kappa statistics. *Public Health Nutr.* 2003;6:313-21.
27. de Pinho L, Moura P H, Silveira MF, et al. Development and validity of a questionnaire to test the knowledge of primary care personnel regarding nutrition in obese adolescents. *BMC Fam Pract.* 2013;14:102-112
28. Malinauskas, BM, Aeby, VG, Overton, RF, Carpenter-Aeby, T & Barber-Heidal, KA survey of energy drink consumption patterns among college students. 2007. *Nutr J*, 6(1):35-41.

CHAPTER 4: ARTICLE 2

Knowledge and perceptions of North-West University rugby players on the timing of protein ingestion

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Running head: Knowledge and perceptions of university athletes on protein timing.

ABSTRACT

Purpose: To determine the knowledge and perceptions of university rugby players on timing of protein intake.

Methods: A descriptive, cross-sectional study with a quantitative and qualitative component was conducted. The study population included 103 male rugby players from a resident university in South-Africa. A self-administered validated knowledge questionnaire on timing of protein intake was completed under supervision. Focus group discussions were conducted to determine the rugby players' perceptions on timing of protein intake.

Results: The overall mean percentage knowledge score on timing of protein intake was $39.8 \pm 13.9\%$ and only 29% scored $\geq 50\%$. Although the first and u/21 teams tended to score higher on the knowledge questionnaire compared to the u/19 teams (42.0 [33.0 - 50.0] vs. 42.0 [33.0 - 42.0] vs. 33.0 [25.0 - 42.0], $p = .06$) knowledge scores was not different between the forwards and the backs. A spearman rank correlation demonstrated an overall weak positive correlation ($r = 0.24$, $p = 0.02$) between weight and knowledge score. The most common perception regarding protein timing was that the best time to consume protein is after training. They concurred that 30 minutes after exercise is important to replenish protein and avoid muscle breakdown. Inter- and intra-reliability of focus group discussions were good ($\kappa = 0.74$ and $\kappa = 0.81$ respectively).

Conclusion: Although North-West University rugby players perceived protein timing to be important, they have poor knowledge regarding protein timing specifically with regards to the type and amount of protein to be consumed. The fact that they knew when to ingest protein (i.e. after exercise/training) and the perception that protein ingestion is important after exercise could be due to the influence of the coaches instructing them to consume protein after exercise and making protein shakes available to them after exercise. These players should therefore be educated on the timing of protein intake.

Keywords: protein timing, university athletes, nutrition knowledge

INTRODUCTION

Rugby is widely considered as one of the most popular sports watched by millions of people worldwide (Lako et al., 2010). In South Africa rugby is regarded as a national sport and is being developed from school level. At universities, rugby players are further being moulded, developed and prepared for the professional league and they often experience pressure to compete at top level (Hale, 2013). Training and competition in addition to academic commitments provide unique challenges to university rugby players (Simiyu, 2010:17), not only in terms of optimal time management, but also in terms of optimal nutrition.

Correct timing of protein intake has been identified as an important component to optimize the adaptive response to both resistance and endurance training (Phillip & van Loon, 2011; Muntenau et al., 2014). Research conducted by Cermak et al. (2014) and McLain et al. (2013) indicated that timely consumption of protein before, during and after exercise has the ability to increase muscle protein synthesis, muscle glycogen restoration, muscle damage repair, muscle size, muscle strength and potentially performance.

Nutrition knowledge and perceptions has been shown to influence what and when athletes eat (Fox et al., 2011). Therefore, to improve nutritional practices via education (Walsh et al., 2013; Strachan et al., 2009) information on the knowledge and perceptions of timing of protein intake of university rugby players can be useful. A number of studies have examined the nutritional knowledge, perceptions and practices of athletes including college/university athletes, rugby players, and even South African rugby players (Duvenage et al., 2015; Strachan et al., 2009).

Various studies supported the lack of knowledge in protein among college athletes (Rash et al., 2008, Torres-McGehee et al., 2012, Fox et al., 2011). National Collegiate Athletic Association (NCAA) Division I track athletes (n = 113) lacked knowledge regarding the roles of protein where 40% (n = 45) believed that the body relies on protein for immediate energy (Rash et al., 2008). In more recent studies, Torres-McGehee et al. (2012), also revealed a lack of nutritional knowledge (54.9%±13.5 mean knowledge score) among 185 university athlete from diverse sport codes including football.

NCAA Division I male athletes (n = 42) actively engaged in strength training perceived their protein needs to be excessively high (2.4 g/kg BW per day) (Fox et al.

2011). These athletes also perceived protein to be the primary source of energy for muscle (Fox et al., 2011). A study conducted on 68 rugby schoolboys in South-Africa indicated that they perceived protein to increase strength (56%), facilitate improved training (25%), promotes weight gain (muscle mass) (63%) and aid recovery (56%) (Strachan et al., 2009). Duvenhage et al. (2015) found that almost half of the 198, u/16 South African rugby players believed their diets to be poor. They had better knowledge about recovery strategies than pre-game meals. Hundred and thirty six (75%) participants identified optimal timing and 109 (56%) knew the ideal macronutrient composition of recovery meals.

Limited research is available on the knowledge and perceptions of university rugby players specifically relating to the timing of protein intake. Therefore, this study aimed to determine the knowledge and perceptions of university rugby players on timing of protein intake.

METHODS

Study design and participants

A descriptive, cross-sectional study with a quantitative and qualitative component was conducted. The study population consisted of 103 male rugby players from a resident university in South-Africa. Participants were included if they were between the ages of 18 - 24 years, enrolled in a degree or diploma at the university, and playing for the universities 1st, u/21A or u/19A team or student provincial 1st, u/21A or u/19A team (e.g. these were students from the university who played for the student provincial team). Of the 103 participants that were included in the study, 36 participants (35%) represented the university (n = 29) or provincial student (n = 7) 1st team, 27 participants (26.2%) played for the university (n = 12) or provincial student (n = 15) u/21A team and the remaining 40 participants (38.8%) represented the university (n = 22) or provincial student (n = 18) u/19A team. According to reported position of play, 59 participants (57.3%) are forwards and 44 participants are backs (42.7 %). The general characteristics of these rugby players are summarized in Table 1. Participants self-reported their weight and height and BMI was calculated by dividing weight by two times their height ($\frac{Weight}{Height^2}$). Ethical approval for the study was obtained from the Health Research Ethics Committee of the Faculty of Health

Sciences, North-West University (NWU-00022-15-S1) and all the participants provided verbal and written informed consent to participate in the study.

Table 1: Subject characteristics (n = 103)

Characteristic	Mean \pm SD	Range
Age (years)	20.4 \pm 1.6	18.0 - 24.0
Weight (kg)	94.9 \pm 13.0	69.0 - 120.0
Height (m)	1.83 \pm 0.1	1.58 - 2.03
BMI (kg/m ²)	28.3 \pm 3.2	21.6 - 37.0

SD = Standard Deviation; kg = kilogram; m² = square meter

Quantitative methodology

The participants completed a previously validated knowledge questionnaire on the timing of protein intake (Swanepoel et al., in review) under supervision in a central venue. The questionnaire included questions on age, weight, height, team, position of play as well as 12 multiple choice questions on timing of protein intake.

Qualitative methodology and how unexpected findings emerged

Seven participants from the university 1st team, university u/21 team, university u/19 team and 1st student provincial team were randomly selected and grouped together in teams to participate in a semi-structured open ended focus group discussion on timing of protein intake. Four focus group discussions in total were conducted by the same facilitator and scribe with experience in conducting focus group discussions. Discussions were conducted according to standard procedures (Krueger, 2009) in an enclosed comfortable venue. The interviews had distinct objectives; however due to the nature of the open ended interview format, participants were allowed to speak freely. This resulted in series of unexpected topics. Data resulting from the first two focus group discussions were analysed by the interviewer and then used to adapt the interview questions (Bernard, 2002). Through this process themes relating to the source of protein intake were revealed and developed. Although the basic structure of the interviews did not change this topic was probed upon. Even though these findings were unexpected it was further explored and included in the results since it was evident that it was important to the participants. This was done in such a way that it did not enforce ideas originated by the researcher or a preconceived hypotheses – a grounded theory approach to data analysis (Denzin & Lincoln, 2006).

Data analysis

Analysis of quantitative results was performed using SPSS, version 22 (SPSS, Chicago, IL, USA). Data were tested for normality with the Shapiro Wilk test. Normally distributed data were reported using means and standard deviations and non-normally distributed data were reported as median and inter-quartile ranges. Knowledge scores were reported as frequency counts and percentages. Mann Whitney U tests were performed to test differences between the respective university and student provincial teams, and Kruskal-Wallis to test knowledge score differences between the 1st, u/21A and u/19A teams. Pearson and Spearman rank correlation coefficients were used to test associations between variables. Statistical significance was accepted as a p-value < 0.05.

Audio-recorded qualitative data were transcribed, captured in ATLAS ti™ version 7 and assessed by means of thematic content analysis using an inductive coding approach (Boeije, 2002; Elo & Kyngäs, 2008). Themes and categories were identified and coded, by the researcher, from the data and summarized in a matrix that facilitates the identification of relationships between and within the different themes (Boeije, 2002). Themes and categories were further explored to gain a deeper understanding of the participants' perceptions on timing of protein ingestion. Finally, conclusions were drawn from the themes and authenticated based on three considerations: 1) credibility of the information; 2) were participant responses a result of probes by the researcher or spontaneously produced by the participants, and 3) potential influence of presence of the researcher. After all conclusions were authenticated a network model was designed to visualise and interpret conclusions.

Data credibility

Data collection after the second interview ended when the researchers established that a set of recurrent themes, relevant to the initial study goal of exploring perceptions of protein intake were identified. No new themes in this regard were evident (thematic saturation). However two more focus group discussions for the two higher teams was conducted as these teams might be different as they had more exposure and have been playing for longer. After the second interview it was evident that the themes did not change for these teams.

Thematic saturation was also ensured relative to the unexpected finding concerning the source of protein intake. Inter- and intra-reliability testing was carried out to test

the reliability of the data. Inter-rater reliability is the agreement of data coded by different coders and was done by two of the researchers well acquainted with the study purpose and coding program. Intra-rater reliability is the agreement of data coded twice by the same coder 3-7 days apart and was done by one of the researchers, 5 days apart. Determining inter- and intra-rater reliability is important in order to determine if the qualitative data collected in the study are consistently coded. Inter- and intra-reliability was determined using the Cohen's kappa, a chance corrected measure that allows for mutual agreement and disagreement of both chance and rating correlation (Anderson et al., 2001). The kappa values were interpreted according to McHugh (2012) where values ≤ 0 indicates no agreement, values from 0.01 – 0.20 shows very little agreement, 0.21 – 0.40 is fair agreement, 0.41 – 0.60 indicates moderate agreement, 0.61 – 0.80 is substantial agreement, and 0.81 – 1.00 is almost perfect agreement.

RESULTS

Knowledge on timing of protein intake

The overall mean percentage knowledge score of the participants was $39.8 \pm 13.9\%$ and only 29% of the participants scored 50% or higher. Individual knowledge scores ranged from a score of one out of 12 (8%) to 10 out of 12 (83%). Table 2 provides a summary of the frequency and distribution of individual knowledge scores in the total group and between age-group teams (i.e. university and student provincial teams combined for u/19, u/21 and 1st teams, respectively).

Since there were no significant age, weight or height differences between the university versus provincial 1st, u/21A or u/19A teams, the respective age group teams were combined to explore differences between the 1st, u/21A and u/19A teams. The overall mean percentage knowledge scores for the combined 1st, u/21/A and u/19A teams are summarized in Figure 1. The first and u/21 teams tended to score higher compared to the u/19A teams ($42.0[33.0 - 50.0]$ vs. $42.0[33.0 - 42.0]$ vs. $33.0[25.0 - 42.0]$ respectively $p = .06$) (Figure 1). In the 1st teams 38.9% scored $\geq 50\%$ while 25.9% of u/21 team participants and 22.5% of u/19 team participants scored $\geq 50\%$

Table 2: Frequency and distribution of individual knowledge scores

Knowledge score out of 12 (%)	Number of participants obtaining specific test score			
	1 st team	u/21	u/19	Total group
1/12 (8%)	0	1	2	3
2/12 (17%)	1	1	1	3
3/12 (25%)	3	5	9	17
4/12 (33%)	9	4	12	25
5/12 (42%)	9	9	7	25
6/12 (50%)	6	6	4	16
7/12 (58%)	5	1	3	9
8/12 (67%)	0	0	2	2
9/12 (75%)	2	0	0	2
10/12 (83%)	1	0	0	1
11/12 (92%)	0	0	0	0
12/12 (100%)	0	0	0	0

When knowledge score differences were explored between the forwards and the backs no significant differences were found (42.0 [42-50] vs. 33.0 [25-42]; $p = 0.22$)

A percentage score for each individual question was calculated based on the number of participants who answered the respective question correctly. Table 3 provides a summary of all 12 questions together with the performance per total group as well as the three age group teams. These scores ranged from 11.7% (Question 11) to 87.4% (Question 10) (Table 3). Half of the participants correctly identify what timing of protein intake entails (Question 1), and the majority (81.6%) knew which athletes can benefit from optimal protein timing (Question 2).

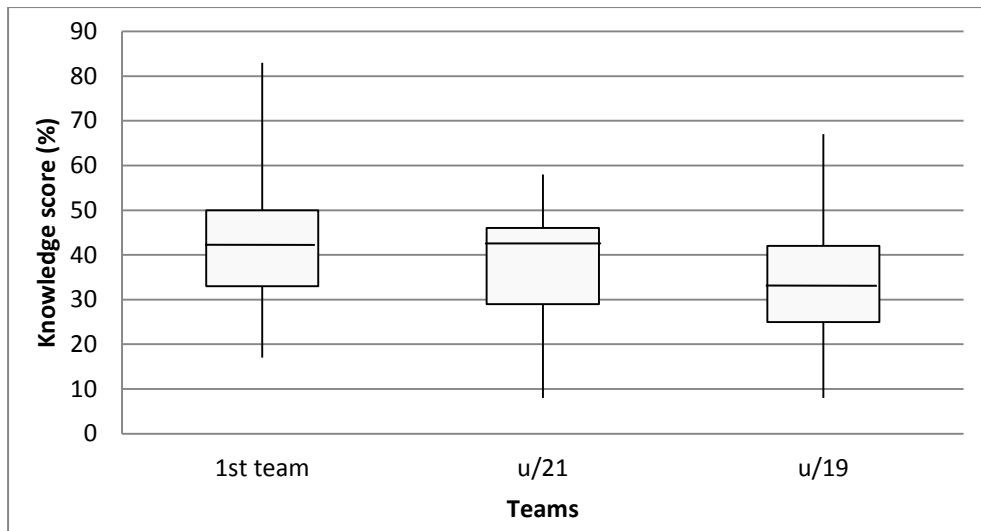


Figure 1: Comparison of the knowledge scores between the 1st, u/21A and u/19A teams

The majority of participants also correctly identified optimal time for protein consumption after exercise (87.4%, Question 10). They were however not that knowledgeable with the role and benefits (Questions 3 and 8) nor amount and source of optimal protein consumption before, during or after exercise (Questions 4, 6 and 11, Table 3). Judged by the percentages in the table, the 1st team performed the best for the majority of the questions (Table 3).

The Spearman rank correlation demonstrated an overall weak positive correlation ($r = 0.24$, $p = .02$) between weight and knowledge score (Figure 2).

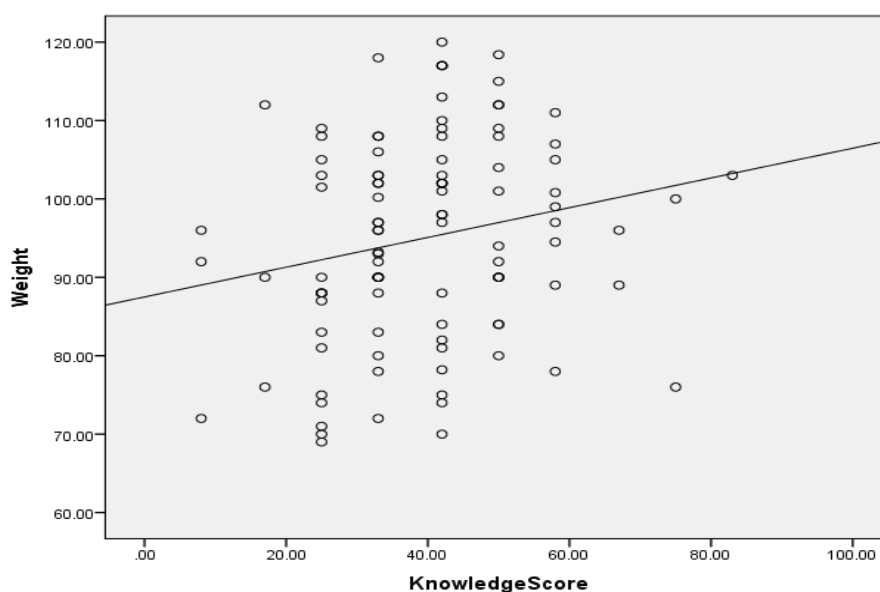


Figure 2: Association between weight and knowledge score (n=103)

Table 3: Percentage participants answering each of the questions correctly

Question	Question	Mean Total Percentage	1 st team	u/21	u/19
1	In a sport nutrition context, which one of the following statements explains 'the timing of protein intake' the best?	52.4	58.3	51.8	47.5
2	Which of the following athletes can benefit from optimal timing of protein ingestion?	81.6	83.3	88.8	72.5
3	Which one of the following is not a potential benefit of eating protein at the right time in context to your training session:	33.5	44.4	18.5	37.5
4	The recommended amount of protein to consume <i>before</i> <u>resistance</u> exercise in combination with carbohydrate for an 80kg athlete is:	15.5	22.2	11.1	12.5
5	Protein ingested <i>before</i> <u>resistance</u> training can potentially:	31.1	50.0	29.6	17.5
6	What is the recommended ratio of carbohydrate-to-protein to consume <i>during</i> exercise?	12.5	13.9	3.7	20.0
7	Protein consumption <i>during</i> exercise can potentially:	46.6	50.0	44.4	45.0
8	The role of protein consumed <i>during</i> <u>resistance</u> exercise is primarily:	17.6	19.4	18.5	15.0
9	The recommended amount of protein to consume <i>after</i> <u>resistance</u> exercise is:	29.1	30.6	29.6	27.5
10	The best time to consume protein <i>after</i> exercise or an event is?	87.4	83.3	96.2	82.5
11	Which one of the following protein sources has the greatest effect on muscle protein synthesis when consumed <i>after</i> <u>resistance</u> exercise?	11.7	13.9	11.1	10.0
12	Protein consumption in combination with carbohydrates <i>after</i> exercise is important to:	58.6	63.9	59.3	52.5

Results (Qualitative)

A total of 27 randomly selected participants from all six teams were included in four focus group discussions. The inter-rater reliability of the coded qualitative data was good ($\kappa = 0.74$) and demonstrated a substantial amount of agreement between the two coders. Intra-rater reliability ($k = 0.81$) was very good, demonstrating an almost perfect agreement between the first and second attempt at coding the same transcript (McHugh, 2012). Eight themes with 54 categories were identified and are summarized in Table 4.

Table 4: Summary of primary themes with their respective categories

Themes	Categories
Perceived definition	3 categories: What and when, when around training or when for benefits
Time, place and person of first encounter	8 categories: Day of study, friends and family, school, tertiary institution, other sport codes, coaches or supplement companies, implementation strategies.
Perceived best time of protein intake	5 categories; In the morning, before and during bedtime, before training, during training, after training.
Reason for perceived best time	3 categories: Before and during bedtime for muscle growth, before and during training to prevent fatigue and after training to recover micro-damage in muscles
Perceived benefits and importance of protein timing	8 categories: Size, recovery, performance, muscle endurance, energy source, appearance, amount is more important, carbohydrates is more important
Practise of protein timing and reason thereof	11 categories: Reason for practise, implementing strategies, no routine, time of season, barriers, advice, morning, before bedtime, before training, during training, after training.
Source of protein consumption	5 categories: Food, supplements, source consumed in the morning and evening, best source to consume around, supplement availability
Source of information on protein timing	11 categories: Internet, body builders, coaches, health professionals, fellow team mates, professional teams, old wife tales, supplement labels, supplement reps, supplement sources, information reliability.

The theme “Perceived definition” emerged from the participants’ response to the prompt “protein timing” and the perceived meaning they connected to this concept. Three categories emerged

from this theme (Table 4). A number of participants commented on what they perceive protein timing to be or connected the definition simply to when protein should be consumed.

"Timing your protein intake."

"The best time to consume protein."

"When to eat."

Some participants perceived protein timing as a specific time when protein should be consumed whilst others perceived it as a specific benefit gained. Some participants even combined the concept that it is the specific time when protein is consumed around training sessions and throughout the day.

"After training, before or after training."

"Directly after gym or just before going to sleep."

"It's the right time to consume it, to gain the best results."

"When your muscles are sore."

"When protein should be consumed, after or before training or right through the day, in the evening or if you wake up in the morning."

With regards to the time, place and person of first encounter of the concept, it was interesting to learn that many of these players were not familiar with the term 'Protein timing' although eight categories emerged from the theme (Table 4). The majority of participants reported that they only heard the concept for the first time on the day of the data collection whilst some heard it as early as secondary school (Table 4).

"I have never heard of the term protein timing before."

"I must be honest, 2 minutes ago, in the quiz, I did not know what it meant."

"I think the word is a given, but I've heard about the term for the first time today in the test."

"When I was still in school, grade 10."

The perceived best time of protein intake included five distinct categories (Table 4). The most common perception was that the best time of intake is after training. They furthermore perceived that it is important to specifically consume protein approximately 30 minutes after exercise.

"20 - 30 minutes after training."

"Half an hour after training."

"Within 45 minutes after training is the optimal time to take it, I have read and watch things on YouTube that this window is actually much bigger."

The participants' reasons to support their perceptions regarding the best time of protein consumption included 3 categories (Table 4).

“Before you get tired take protein to prevent muscle fatigue like before or during exercise.”

“It helps for recovery if you consume protein shakes after training.”

“Consume protein half an hour after exercise to recover micro-tears.”

“When you sleep protein synthesis occurs so take protein before you go to sleep.”

“Your body absorb protein better when you sleep and then you grow better.”

“Your body recovers when you sleep.”

However some of the participants felt that the best time to consume protein is before bed.

“It is important to consume protein before you go to sleep.”

“Just before you go to sleep.”

“I think in the evenings.”

“If you consume protein prior to bed time your body absorb it better. It's like for a baby, they drink milk which is protein and then go to sleep and that's why they grow rapidly.”

This resulted in another curious opinion that protein should be consumed during bedtime.

“First 45 minutes of sleep is vital, I wake up during the night and take a protein shake and then sleep on and I will grow.”

In general participants perceived the timing of protein intake as important and distinct categories emerged from the theme ‘perceived benefits and importance of protein ingestion’ (Table 4). The majority of participants believed that the correct timing of protein intake contribute to building muscle which they perceive to be important if you want to play for a professional or national team.

“If you are a rugby player in South-Africa, the bigger you are the quicker you will play Springbok.”

“If you are bigger in size it will be beneficial for performance.”

“To recover your muscles.”

“To prevent muscle breakdown.”

There were however some of the participants who did not think the time of protein intake is that important but that the amount of protein or timing of carbohydrate timing are more important.

“It's not about the time when you consume protein, but about the amount of protein.”

“I think protein is very important but the timing isn't as important as say the timing of carbohydrate intake.”

“You need more carbohydrates than protein, 8g protein for 16g carbs, 2 to 1.”

The theme ‘Practice of protein timing and reason thereof’ provided very valuable and perhaps novel insight regarding protein timing practices. Although this theme touches on a practice and not a perception per se, this information can help to contextualize some of their perceptions. Eleven categories emerged from this theme (Table 4). Despite the fact that the majority of participants professed protein intake after training to be important, judged by the

responsiveness and feedback from the players it seems that only a few participants consciously implemented a protein timing strategy.

“Lots of these guys don’t know about protein timing but actually it’s forced fed to us that you must have protein after things because when you finish gym there is protein there, everybody takes their protein.”

“The idea have been actually given to us without us knowing because protein is available only in that 45 minute window say after training.”

“We are well led by coaches, for instance when to eat, but we don’t know it ourselves.”

“If you forget your shaker it’s a fine.”

“We get protein after gym and stuff.”

“Shakes are available after games, you just take it.”

Not all the players felt obliged to consume protein at a certain time or focus on protein timing all the time.

“I’d rather be a fast guy than a well build guy so my focus is elsewhere, that’s why protein and protein timing is not really a big thing to me.”

“Some of us are not that interested as others.”

“I’m not that big on supplements, so this is all new to me.”

The university 1st team reported that they are much more focused on nutrition in-season compared to pre-season. In-season they are provided with a sport scientist whom guide them step by step and supply them with supplements. The fact that supplements are readily available after training sessions added to their nonchalant attitude towards the issue. They don’t know how long after training they should consume protein; they just do so because they are told to so without understanding why. A number of the participants appeared to be more knowledgeable and eager to consciously implement protein timing strategies. They also offered reasons for why they are not implementing it.

“We only have 20 minutes break, how should we prepare veggies and steak in 20 minutes and get to training or class, then we just grab whatever because we are hungry.”

“Our training time is at 1pm to 2pm because of the students who have class, so it’s difficult to eat lunch when lunch is meant to be eaten and then we train 5pm to half past 7 and that’s when we should have dinner. We gym, at 11am after which I have a protein shake and then when I get home I’m full to eat again, so sometimes we can’t even get proper food in.”

Even though this study focused on the perceptions regarding timing of protein, an unexpected theme around protein source arose from which 5 categories emerged (Table 4). This included the source of protein chosen by these participants and this was investigated further. Participants considered eggs, chicken, fish, red meat, nuts and milk to be ideal protein sources. Food consumption in the morning mostly included oats and eggs. Meat, eggs, tuna and protein

shakes were commonly reported in the evening before bed. One participant reported that chicken helps him to lose weight. Food, not necessarily protein only, was typically consumed pre-match, whilst supplements were consumed afterwards.

"I'm not great on breakfast but I try to eat eggs and then at lunch I always eat steak with eggs, with a lot of eggs. Then in the evening I try to eat chicken."

"As a team we have a pre-match meal where the coach provides us with a chicken pasta."

"After a match we are provided with protein shakes and before the match we will have a banana or muffin."

"You will not have a steak before a match, you will feel heavy."

"Chicken is the best, I consumed it whenever I could, all of the time, loads of it, to lose weight."

An interesting observation from participants from younger teams was the perception that protein consumed prior to sleeping will make you anabolic and contribute to muscle building or recovery.

"Tuna, tuna makes your body anabolic."

"Before you go to sleep in the evening you have to consume a small portion of protein, like eggs, to recover your body while you sleep."

The source of information on the timing of protein intake was a theme that emerged from the players wanting to justify why they do certain things by telling the interviewers where they got the information from. The majority of participants received their information from a source on the internet. You Tube videos were the most popular source to gather information and very few of them read factual pages. They idealize bodybuilders and believe what they practise and advise because they see results. These athletes believed that size equals strength and performance.

"I just searched You Tube to see what guys do that gym."

"If you are a resident in a campus res, it is very popular to use YouTube."

"Sometimes when I search Facebook, I will follow links, that's where I learn."

"The proof is in the pudding, with this protein thing, cause you see those hectic body builders, when they do it properly they take what the text book says and you can see the results, so we see them and think it must work."

A common perception is also that coaches are a source of information/guidance.

"We just trust the system."

"We follow what the coaches tells us."

"We follow coaches blindly."

It is possible that coaches contribute to the perception of these participants around the timing of protein intake, through being a source of information by implementation of strategies.

An important function of qualitative research is to hypothesise for further study (Co & Perrin, 2005). Figure 3 represents an outline of our theory. We theorise that the perceptions uncovered in the present study may come from direct experience through implementation led by the coaches (a strong influential source of information) without it necessarily being supported by education on the topic (Figure 3). Theoretical implementation of these protein timing strategies could have revealed the importance of protein timing and therefore inspired participants to seek information on this topic (via You Tube). Indeed, participants reported that their main source of information is the internet and specifically “YouTube” which could possibly be unreliable but contribute to their perceptions.

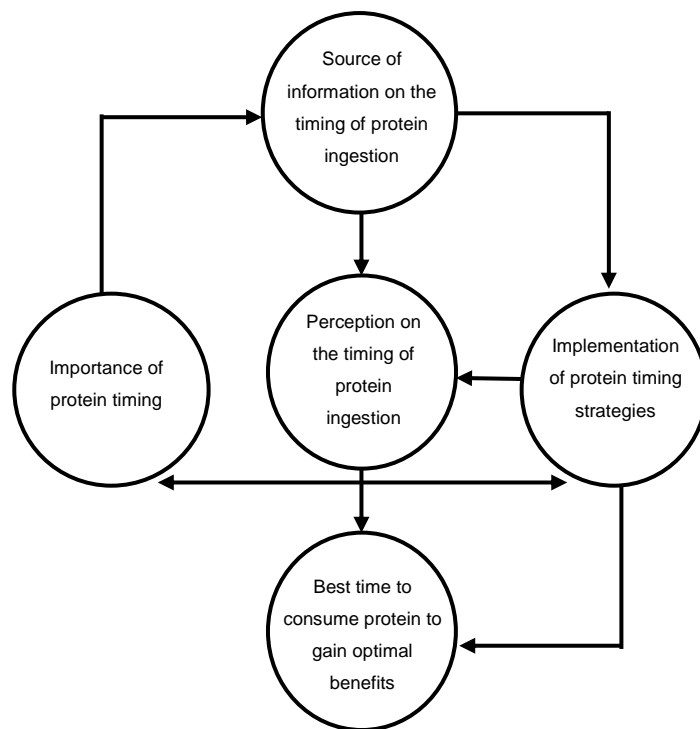


Figure 3: Schematic outline of theory

DISCUSSION

The present study examined the knowledge and perceptions of university rugby players on the timing of protein intake. Mean overall knowledge score ($39.8 \pm 13.9\%$) of this group of university rugby players was poor and only 29% of the participants scored $\geq 50\%$. Half of the participants correctly identified *what* timing of protein intake entails and the majority of players perceived the concept as being important. The focus group discussions further revealed that the majority of participants indicated that their first encounter of the term “protein timing” was only on the day

the knowledge questionnaire was administered. The participants also demonstrated insufficient knowledge regarding the specific type and amount of protein to consume around training. Torres-McGehee et al. (2012) supported the poor knowledge score revealed in this study when he determined the knowledge score of 185 university athletes, including football players to be inadequate ($54.9\% \pm 13.5$). Knowledge was poor for all domains (macro- and micronutrients, supplements related to performance, weight management and eating disorders, as well as hydration). The section on macro- and micronutrients (including protein) received the lowest mean knowledge score for all participants (Torres-McGehee et al., 2012).

The first and u/21 teams tended to score higher compared to the u/19A teams. One reason could be that these participants revealed in the focus group discussion that they are guided by a sport scientist in-season to implement optimal protein timing strategies. Through implementation, encouraged by the sport scientist and coaches, these players gained knowledge. Furthermore participants from the u/19 teams had a perception that once they reach higher teams they will receive the benefits of protein timing education.

Based on the knowledge questionnaire, 87.5% of the participants correctly answered that the best time to consume protein *after* exercise or an event is approximately 30 minutes after exercise. This was supported by results from the focus group discussion where the majority of the players also had the perceived idea that the best time of protein consumption after training is approximately 30 minutes after training. Although the majority of participants seem to know when to consume protein after training for optimal benefits, only a few reported to implement this timing strategy. These findings are supported by Walsh et al. (2011) who highlighted disparities between knowledge and practices in 203 male senior schoolboy rugby players. In that study 68% of athletes knew they should eat immediately after exercise but only 61.6% indicated that they actually ate within half an hour post exercise (Walsh et al., 2011). Despite the fact that a positive attitude toward nutrition was observed in these players, poor nutritional knowledge and dietary practices were present (Walsh et al., 2011).

Participants in the present study were knowledgeable regarding the type of athletes whom will benefit from applying protein timing strategies (81.6%) and a number of players professed in the focus group discussion that the correct timing will contribute to recovery and muscle building. Another perception that came out is that greater/bigger muscles are an important consideration to be included in a professional or national team. This may in part explain the overall weak positive correlation ($r = 0.24$, $p = 0.02$) between weight and mean knowledge score. The drive to deliberately increase muscle mass may have motivated some players to do research on the topic. These participants were however unfamiliar with the role (17.5%) and benefits (31.1%; 35.5%) of protein timing as well as the source (11.7%) and amount (15.5%; 12.5%) of protein to take as part of the timing strategy. Of note is the fact that the validation study of the knowledge

questionnaire (Swanepoel et al., in review) demonstrated that the question on the amount of protein was considered “too difficult” based on the item difficulty index (<10%). It was indicated that the deletion of this question and the question on the source of protein consumed after training would strengthen consistency. The researchers however decided not to remove these questions as they considered it an important factor when testing knowledge on the timing of protein intake.

The focus group discussions also revealed an unexpected finding, indicate that the source of protein was an important topic to the participants and they had a lot to say about it. However they were not sure as to what is a good protein source, around training as there were a lot of different ideas and practices. They also regarded the amount of protein to be more important than the time of protein consumption. Supporting these findings is Burkhart, (2010) who determined the nutrition knowledge in talented adolescent athletes including rugby players and reported that rugby players had indicated poor knowledge on the amount and source of pre-event food intake (0%), during event food and fluid intake (17.86%) and the amount of recovery food (14.29%).

The majority of participants received their information on the internet and “You Tube” was the most popular source of information while few participants read factual pages. It seemed as though coaches also have an influence on the practice of these participants through the strategies that are being implemented. A study conducted among 145 NCAA Division I athletes, aiming to investigate the sources of supplement information. The study revealed that the internet (79.0%) was the most popular source of information (Malinauskas et al. 2007) while Hale, (2012) determined, after evaluating the nutritional habits and knowledge of 45 freshman senior football players, that over 46% of athletes rely on their strength training coaches for guidance. Burns et al., (2004) supported these findings after evaluating the role of athletic trainers and dietitians in nutrition counseling of 228 university athletes (including football players). Results revealed 39% of the athletes received information from strength training coaches, 23% from athletic trainers and only 14% from dietitians.

CONCLUSION

According to the knowledge of the authors this is the first study to examine the knowledge and perceptions of university rugby players on the timing of protein intake. The knowledge of these participants are poor, and even more so in the younger teams. The areas that was identified that need improvement are their knowledge on the amount and source of protein to consume around training, also on the benefits gained from consuming protein at the correct time. The fact that the players knew when to ingest protein (i.e. after exercise/training) and the perception that protein ingestion is important after exercise could be due to the influence of the coaches

instructing them to consume protein after exercise and making protein shakes available to them after exercise. These players should therefore be educated on the timing of protein intake.

STRENGTHS AND LIMITATIONS

Qualitative research allows for gaining a clearer and deeper understanding of the targeted market. With a clear comprehension of the knowledge, perceptions and practices of athletes, researchers can reach out to the population and ensure correct information are conveyed to improve their knowledge on the topic and affect their perceptions.

The team involved in conducting this study consisted of a diverse team with expertise ranging from sports nutrition to conducting focus group discussions.

A possible limitation was that the research quality is dependent on the background and individual skills of the researchers, and can be more easily influenced by the researcher's personal prejudices. However the researchers did put certain strategies in place to ensure the rigor of the data.

The sample size in the present study was based on convenience sampling and all the available rugby players from NWU playing for the 1st, u/21A and u/19A teams were included in the study. Furthermore, although rugby players were randomly selected from all six teams, not all the players volunteered to take part in the focus group discussion. Only four focus group discussions were conducted (one with the NWU 1st team, one with the student provincial 1st team, one for the u/19 A teams with players from NWU and student provincial combined and one for u/21 A teams with players from NWU and student provincial combined). It is advisable to conduct a focus group for each NWU team and for each student provincial team to determine if there are differences in the perceptions of NWU teams and student provincial teams from each respective age group.

RECOMMENDATIONS FOR FUTURE STUDIES

Future research should include a larger sample size and also include student rugby players from other universities in order to determine knowledge and perceptions of university rugby players, and not just NWU rugby players. Future studies could also include in-depth interviews and focus group discussions with coaches, managers and trainers to capture their perceptions. Future studies could focus on practises and supplements to expand the questionnaire and make it more comprehensive.

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REFERENCES

- Alaunyte, I., Perry, J. L., & Aubrey, T. (2015). Nutritional knowledge and eating habits of professional rugby league players: does knowledge translate into practice? *Journal of the International Society of Sports Nutrition*, 12(1),18.
- Anderson, T., Rourke, L., Archer, W., & Garrison, R. (2001). Assessing teaching presence in computer conferencing transcripts. *Journal of the Asynchronous Learning Network*, 5(2).
- Barzegari A, Ebrahimi M, Azizi M., & Ranjbar K. (2011). A study of nutrition knowledge, attitudes and food habits of college students. *World Applied Sciences Journal*, 15(7), 1012-1017.
- Bernard, H.R. (2002). *Research Methods in Anthropology: Qualitative and Quantitative Approaches*. 3rd ed. Walnut Creek: Altamira Press.
- Boeije, H. (2002). A purposeful approach to the constant comparative method in the analysis of qualitative interviews. *Quality and quantity*, 36(4), 391-409.
- Burkhart, S. J. (2010). *Assessment of nutritional knowledge and food skills in talented adolescent athletes: a thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition at Massey University, Palmerston North, New Zealand* (Doctoral dissertation).
- Cermak, N. M., de Groot, L. C., Saris, W. H. & van Loon, L. J. (2012). Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *The American journal of clinical nutrition*, 96(6), 1454-1464.
- Co, J.P. & Perrin, J.M. (2002). *Qualitative Research and Ambulatory Pediatrics*, 5(3):129–130.
- Cole, C.R., Salvaterra, G.F., Davis, J.E., Jr., Borja, M.E., Powell, L.M., Dubbs, E.C., & Bordi, P.L. (2005). Evaluation of dietary practices of National Collegiate Athletic Association Division I football players. *Journal of Strength and Conditioning Research*, 19:490–494.
- Denzin, N.K., & Lincoln, Y.S., editors. (2006). *The Sage Handbook of Qualitative Research*. 3rd ed. Sage Publications.
- Duvenage, K.M., Meltzer, S.T., & Chantler, S.A. (2015). Initial investigation of nutrition and supplement use, knowledge and attitudes of under-16 rugby players in South Africa. *South African Journal of Sports Medicine*, 27(3):67-71.

- Fox, E.A., McDaniel, J.L., Breitbach, A.P., & Weiss, E.P. (2011). Perceived protein needs and measured protein intake in collegiate male athletes: an observational study. *Journal of the International Society of Sports Nutrition*, 8(9).
- Hale, M. (2013). Nutritional Habits & Knowledge in the Division I Collegiate Football Player. *All Graduate Plan B and other Reports Paper*, 283.
- Krueger, R.A., & Casey, M.A. (2009). Focus groups: A practical guide for applied research. 4th ed. Thousand Oaks, CA: Sage.
- Lako, J.V., Sotheeswaran, S., & Christi, K S. (2010). Food habits and nutritional status of Fiji rugby players. *World Academy of Science, Engineering and Technology*, 68, 742-7.
- Malinauskas, B. M., Aeby, V. G., Overton, R. F., Carpenter-Aeby, T., & Barber-Heidal, K. (2007). A survey of energy drink consumption patterns among college students. *Nutrition Journal*, 6(1), 35-41.
- McHugh, M.L. (2012). Interrater reliability: the kappa statistics. *Biochemia Medica*, 22(3), 276-282.
- McLain, T.A., Escobar, K.A., & Kerksick, C.M. (2015). Protein applications in sports nutrition – part I: Requirements, quality, source, and optimal dose. *Strength and Conditioning Journal*, 37(2), 61-71.
- Munteanu, A. M., Manuc, D., Caramoci, A., Vasilescu, M., & Ionescu, A. (2014). Nutrition timing in top athletes. *Medicina Sportiva: Journal of Romanian Sports Medicine Society*, 10(3), 2357.
- Phillips, S.M., & Van Loon L.J. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *Journal of Sports Science*, 29(1):29-38.
- Potgieter, S. (2013). Sport nutrition: A review of the latest guidelines for exercise and sport nutrition from the American College of Sport Nutrition, the International Olympic Committee and the International Society for Sport Nutrition. *South African Journal of Clinical Nutrition*, 26(1):11-13.
- Potgieter, S., Visser, J., Crouwkamp, I., Markides, M., Nascimnto, J., & Scott, K. (2014). Body composition and habitual and match-day dietary intake of the FNB Maties Varsity Cup rugby players. *South African Journal of Sports Medicine*, 26(2):35-43.
- Rash, C. L., Malinauskas, B. M., Duffrin, M. W., Barber-Heidal, K., & Overton, R. F. (2008).

Nutrition-related knowledge, attitude, and dietary intake of college track athletes. *Sport Journal*, 11(1), 48-54.

Rodriquez N.R., DiMarco N.M., & Langley S. (2009). Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the American Dietetic Association*, 109(3), 509-527.

Slater G. & Phillips S.M. (2011). Nutrition guidelines for strength sports: sprinting, weightlifting, throwing events, and bodybuilding. *Journal of Sports Science*, 29(S1), S67-S77.

Strachan, K. (2009). Observational descriptive study depicting current perceptions and usage practices of nutritional supplements amongst adolescent rugby-playing school boys from the Kwazulu Natal region.

Torres-McGehee, T.M., Pritchett, K.L., Zippel, D., Minton, D.M., Cellamare, A., & Sibilia, M. (2012). Sports nutrition knowledge among collegiate athletes, coaches, athletic trainers, and strength and conditioning specialists. *Journal of Athletic Training*, 47(2), 205–211.

Walsh, M. Cartwright, L. Corish, C. Sugrue, S., & Wood-Martin, R. (2011). The body composition, nutritional knowledge, attitudes, behaviours, and future education needs of senior schoolboy rugby players in Ireland. *International Journal of Sport Nutrition and Exercise Metabolism*, 21 (5), 365-376.

CHAPTER 5: SUMMARY AND CONCLUSION

5.1 Summary

Limited literature is available specifically on the knowledge and perceptions of university athletes on protein timing, therefore this MSc dissertation aimed to determine the knowledge and perceptions of North-West University rugby players on timing of protein intake. Part one of this dissertation included the development and validation of a reliable questionnaire to determine knowledge of timing of protein intake in university athletes. A 12-item valid and reliable knowledge questionnaire was developed with good item difficulty and good strength of consistency. The questionnaire is reliable at group level for agreement and association. On individual level the questionnaire showed limited bias and limited error and strong association.

During part two of this dissertation, this valid knowledge questionnaire was completed by 103 North-West University rugby players. In line with the hypotheses, the main overall knowledge score of North-West university players on timing of protein intake was poor ($39.8 \pm 13.9\%$) and only 29% of these players scored 50% or higher. Furthermore, these players' knowledge specifically regarding the role and benefits, as well as the optimal source and amount of protein to consume before and after exercise were poor (ranged from 11.7 – 35.5%). Although the overall mean knowledge score was low, the majority of participants correctly identified the optimal time to consume protein after training (question 10) and knew which athletes will benefit from ingesting protein at the right time (question 2). During validation of the knowledge questionnaire, these two questions also obtained the highest item difficulty scores (i.e. were answered correctly by the majority of participants in the validation study) and were regarded as easy. Although the participants perceived timing of protein intake to be important, source of protein consumed by these participants was discussed numerous times followed by the source of information they rely on. The most common perception specifically regarding timing of protein intake was that the best time to consume protein is after training. The 30-minute period following exercise specifically was perceived as an important period to replenish protein and avoid muscle breakdown.

Poor knowledge scores relating to the source and amount of protein intake could be a result of information obtained from unreliable sources on the internet (YouTube). The fact that they knew when to ingest protein (i.e. after exercise/training) and the perception that protein ingestion is important after exercise could be due to the influence of the coaches instructing them to consume protein after exercise and making protein shakes available to them after exercise.

5.2 Strengths and limitations

The team involved in this MSc dissertation consisted of a diverse team with expertise ranging from sports nutrition to conducting focus group discussions and validating questionnaires.

The strengths of the validation study included the fact that the validation study was conducted in a population (i.e. male university hockey and cricket players) that closely represents the population of the cross sectional study (i.e. male university rugby players). Qualitative data were collected by means of semi-structured focus group discussions that were all conducted according to standard procedures by the same facilitator and scribe with experience in conducting focus group discussions and analysing qualitative data by means of ATLAS Ti.

Determining the knowledge of these athletes could have been better addressed by a large scale study. Only recruiting athletes specifically from North-West University are a limitation since this sample may not be representative of the general university athlete population. Future research should include student rugby players from other universities to compare knowledge in athletes at different universities. A stronger technique could have been used to determine content validity of the questionnaire therefore future studies could make use of techniques such as Delphi. Future studies could also add questions around practices and supplement use of athletes to make this a more comprehensive questionnaire. In addition, in-depth interviews and focus group discussions with coaches, managers and trainers of the representative teams can be included to capture their perspective as well.

5.3 Conclusion and recommendations

It is concluded that the newly developed questionnaire is valid and reliable. This dissertation further concludes that although North-West University rugby players perceived protein timing to be important, they have poor knowledge regarding protein timing specifically with regards to the type and amount of protein to be consumed at specific times. The fact that they knew when to ingest protein (i.e. after exercise/training) and the perception that protein ingestion is important after exercise could be due to the influence of the coaches instructing them to consume protein after exercise and making protein shakes available to them after exercise. These players should therefore be educated on the timing of protein intake.

CHAPTER 6: BIBLIOGRAPHY

Ahrendt, D.M. 2001. Ergogenic aids: Counseling the athlete. *American family physician*, 63(5):913-922.

Alaunyte, I., Perry, J.L. & Aubrey, T. 2015. Nutritional knowledge and eating habits of professional rugby league players: does knowledge translate into practice? *Journal of the International Society of Sports Nutrition*, 12(1):18.

Alghannam, A.F. 2011. Carbohydrate–protein ingestion improves subsequent running capacity towards the end of a football-specific intermittent exercise. *Applied physiology, nutrition, and Metabolism*, 36(5):748-757.

Alford, S. 2004. Nutritional knowledge and dietary habits of professional and semi-professional football players. MPhil/PhD Thesis University of Liverpool (Chester College of Higher Education).

American College of Sports Medicine, American Dietetic Association, Dietitians of Canada. 2009. Nutrition and athletic performance: joint position statement. *Medicine and science in sports and exercise*, 41(3):709-731.

American Dietetic Association, Dietitians of Canada. American College of Sports Medicine. Nutrition and athletic performance. 2000. *Journal of the American Dietetic Association*, 100:1543-46.

Anderson, L.L., Tufekovic, G., Zebis, M.K., Cramer, R.M., Verlaan, G., Kjaer, M., Suetta, C., Magnusson, P. & Aarard, P. 2005. The effect of resistance training combined with timed ingestion of protein on muscle fibre size and muscle strength. *Metabolism*, 54:151-156

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.

Betts, J.A., Toone, R.J., Stokes, K.A. & Thompson, D. 2009. Systemic indices of skeletal muscle damage and recovery of muscle function after exercise: effect of combined carbohydrate-protein ingestion. *Applied physiology, nutrition, and metabolism*, 34(4):773-784.

Betts, J.A. & Williams, C. 2010. Short-Term recovery from prolonged exercise. *Sports Medicine*, 40(11):941-959.

Bird, S.P., Tarpenning, K.M. & Marino, F.E. 2006. Independent and combined effects of liquid carbohydrate/essential amino acid ingestion on hormonal and muscular adaptations following resistance training in untrained men. *European journal of applied physiology*, 97:225–238.

Blomstrand, E., Hassmen, P., Ekblom, B. & Newsholme, E.A. 1991. Administration of branched-chain amino acids during sustained exercise - effects on performance and on plasma concentration of some amino acids. *European journal of applied physiology and occupational physiology*, 63(2):83-88.

- Bloomer, R.J., Davis, P.G., Consitt, L.A. & Wideman, L. 2007. Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *International journal of sports medicine*, 28(1):21-25.
- Bloomer, R.J., Goldfarb, A.H., Wideman, L., McKenzie, M.J. & Consitt, L.A. 2005. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *The journal of strength and conditioning research*, 19(2):276-285.
- Bohé, J., Low, A., Wolfe, R.R. & Rennie, M.J. 2003. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *The journal of physiology*, 552(1):315-324.
- Borsheim, E., Tipton, K.D., Wolf, S.E. & Wolfe, R.R. 2002. Essential amino acids and muscle protein recovery from resistance exercise. *American journal of physiology, endocrinology and metabolism*, 283:E648– E657.
- Bucci, L.R. & Unlu, L.M. 1999. Proteins and amino acid supplements in exercise and sport. In: *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*. J.A. Driskell and I. Wolinsky, eds. Boca Raton, FL: CRC Press, pp.191–212.
- Burns, R.D., Schiller M.R., Merrick M.A. & Wolf K.N. 2004. Intercollegiate student athlete use of nutritional supplements and the role of athletic trainers and dietitians in nutrition counseling. *Journal of American Dietetic Association*, 104:246-249.
- Burd, N.A., Tang, J.E., Moore, D.R. & Phillips, S.M. 2009. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. *Journal of applied physiology*, 106(5):1692-1701.
- Burke, E. 2003. Optimal Muscle Performance and Recovery: Using the Revolutionary R4 System to Repair and Replenish Muscles for Peak Performance. *Penguin*. 115-118
- Burke, L.M. & Hawley, J.A. 2006. Fat and carbohydrate for exercise. *Current opinion in clinical nutrition & metabolic care*, 9(4):476-481.
- Burke, L.M., Winter, J.A., Cameron-Smith, D., Enslin, M., Farnfield, M. & Decombaz, J. 2012. Effect of intake of different dietary protein sources on plasma amino acid profiles at rest and after exercise. *International journal of sport nutrition and exercise metabolism*, 22(6):452.
- Burkhart, S. J. (2010). *Assessment of nutritional knowledge and food skills in talented adolescent athletes: a thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition at Massey University, Palmerston North, New Zealand* (Doctoral dissertation).
- Campbell, B., Kreider, R.B., Ziegenfuss, T., La Bounty, P., Roberts, M., Burke, D. & Antonio, J. 2007. Journal of the International Society of Sports Nutrition. *Journal of the International Society of Sports Nutrition*, 4(8):8.
- Candow, D.G., Chilibeck P.D., Facci M., Abeysekara S. & Zello G.A. 2006. Protein supplementation before and after resistance training in older men. *European journal of applied physiology*, 97:548-556.

- Catsicas, M.E., Lombaard, M., Potgieter, S. & Nel, D. 2014. An Assessment of the Level of Knowledge of Health Professionals on Nutrition and Diabetes Self-Management in Treating Patients with Type I and Type II Diabetes Mellitus in South Africa. *Faculty of Medicine and Health Sciences Department of Interdisciplinary Health Sciences Division of Human Nutrition paper*, 29-30.
- Cermak, N.M., de Groot, L.C., Saris, W.H. & van Loon, L.J. 2012. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *The American journal of clinical nutrition*, 96(6):1454-1464.
- Clarke, E.J. & Wiseman, J. 2000. Developments in plant breeding for improved nutritional quality of soya beans I. Protein and amino acid content. *The journal of agricultural science*, 134(02):111-124.
- Cole, C.R., Salvaterra, G.F., Davis, J.E., Borja, M.E., Powell, L.M., Dubbs, E.C. & Bordi, P.L. 2005. Evaluation of dietary practices of National Collegiate Athletic Association Division I football players. *Journal of strength and conditioning research*, 19:490–494.
- Cribb P.J., & Hayes A. 2006. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. *Medicine and science in sport and exercise*, 38:1918-1925.
- Cribb, P.J., Williams, A.D., Stathis, C.G., Carey, M.F. & Hayes, A. 2007. Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. *Medicine and science in sports and exercise*, 39 (2): 298-307.
- Dideriksen, K., Reitelseder, S. & Holm, L. 2013. Influence of amino acids, dietary protein, and physical activity on muscle mass development in humans. *Nutrients*, 5(3):852-876.
- Di Pasquale, M.G. 2007. Utilization of Proteins in Energy Metabolism. *Sports Nutrition: Energy Metabolism and Exercise*, (3):63.
- Dreyer, H.C., Drummond, M.J., Pennings, B., Fujita, S., Glynn, E.L., Chinkes, D.L. & Rasmussen, B.B. 2008. Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle. *American journal of physiology-endocrinology and metabolism*, 294(2):E392-E400.
- Dunn, M., Eddy, J., Wang, M.Q., Nagy, S., Perko, M. & Bartee, T. 2001. The influence of significant others on attitudes, subjective norms and intentions regarding dietary supplement use among adolescent athletes. *Adolescence*, 36(143):583-591.
- Duthie G.M., Pyne D.B., Marsh D.J. & Hooper S.L. 2006. Sprint patterns in rugby union players during competition. *Journal of strength and conditioning research*, 20:208–214.
- Dwyer, J.T., Stoulorow, K.A. & Orr, R. 1981. Nutrition knowledge test for high school students. *Journal of nutrition education*, 13:93-94
- Egan, B. & Zierath, J.R. 2013. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell metabolism*, 17(2):162-184.

- Foster, L.J., de Hoog, C.L. & Mann, M. 2003. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proceedings of the national academy of sciences*, 100(10):5813-5818.
- Froiland K., Koszewski, W., Hingst, J. & Kopecky, L. 2004. Nutritional supplement use among college athletes and their sources of information. *International journal of sport nutrition and exercise metabolism*, 14:104-120.
- Gabbett, T. J. 2005. Changes in physiological and anthropometric characteristics of rugby league players during a competitive season. *The journal of strength & conditioning research*, 19(2):400-408.
- Gaeini, A.A., Rahnema, N. & Hamedinia, M.R. 2006. Effects of vitamin E supplementation on oxidative stress at rest and after exercise to exhaustion in athletic students. *Journal of sports medicine and physical fitness*, 46(3):458.
- Gleeson, M. 2007. Immune function in sport and exercise. *Journal of applied physiology*, 103(2):693-699.
- Hartman, J.W., Tang, J.E., Wilkinson, S.B., Tarnopolsky, M.A., Lawrence, R.L., Fullerton, A.V. & Phillips, S.M. 2007. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *The American journal of clinical nutrition*, 86(2):373-381.
- 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.
- Hall, J.E. 2010. *Guyton and Hall textbook of medical physiology*. Elsevier Health Sciences. USA
- Hale, M. 2013. Nutritional habits & knowledge in the division I collegiate football player. *All Graduate Plan B and other Reports Paper*, 283.
- Haub, M.D., Wells, A.M., Tarnopolsky, M.A. & Campbell, W.W. 2002. Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men. *The American Journal of Clinical Nutrition*, 76(3):511-517.
- Hayes, A. & Cribb, P.J. 2008. Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training. *Current opinion in clinical nutrition & metabolic care*, 11(1):40-44.
- Hendricks, S., O'Connor, S., Lambert, M., Brown, J., Burger, N., Mcfie, S. & Viljoen, W. 2015. Contact techniques and concussions in the South African under 18 Coca-Cola Craven Week rugby tournament. *European journal of sport science*, 15(6):557-564
- Hoffman, J.R., Ratamess, N.A., Tranchina, C.P., Rashti, S.L., Kang, J. & Faigenbaum, A.D. 2009. Effect of protein-supplement timing on strength, power, and body-composition changes in resistance-trained men. *International journal of sport nutrition*, 19(2):172.

- Hoffman J.R., Ratamess N.A., Kang J., Falvo M.J. & Faigenbaum A.D. 2007. Effects of protein supplementation on muscular performance and resting hormonal changes in college football players. *Journal of sport science and medicine*, 6:85-92.
- Hoffman, J.R., Ratamess, N.A., Kang, J., Falvo, M.J. & Faigenbaum, A.D. 2006. Effect of protein intake on strength, body composition and endocrine changes in strength/power athletes. *Journal of International Society of Sports Nutrition*, 3:12-18.
- Howarth K.R., Moreau N.A., Phillips S.M. & Gibala M.J. 2009. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of applied physiology*, 106:1394-1402.
- Hulmi, J.J., Lockwood, C.M. & Stout, J.R. 2010. Review Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. *Nutrition and metabolism*, 7:51.
- Imamura, H., Iide, K., Yoshimura, Y., Kumagai, R., Oshikata, R., Miyahara, K., Oda, K., Miyamoto, N. & Nakazawa, A. 2013. Nutrient intake, serum lipid and iron status of collegiate rugby players. *Journal of the International Society of Sport Nutrition*, 10:9.
- Ivy, J. 2004. *Summary of electrolytic hydrogen production: milestone completion report*. National Renewable Energy Lab., Golden, CO (US).
- Ivy J.L., Res P.T., Sprague R.C. & Widzer M.O. 2003. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. *International Journal of sport nutrition and exercise metabolism*, 13:382-395.
- Ivy, J.L., Goforth, H.W., Damon, B.M., McCauley, T.R., Parsons, E.C. & Price, T.B. 2002. Early post-exercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *Journal of applied physiology*, 93(4):1337-1344.
- Ivy, J.L. 1997. Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. *Sports medicine*, 24(5):321-336.
- Jacobson, B.H., Sobonya, C. & Ransone, J. 2001. Nutrition practices and knowledge of college varsity athletes: a follow-up. *The journal of strength & conditioning research*, 15(1):63-68.
- Jenkins, D.J., Mirrahimi, A., Srichaikul, K., Berryman, C.E., Wang, L., Carleton, A. & Kris-Etherton, P. M. 2010. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. *The journal of nutrition*, 140(12):2302S-2311S.
- Jeukendrup, A.E. 2010. Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. *Current opinion in clinical nutrition & metabolic care*, 13(4):452-457.
- Jonnalagadda S.S., Rosenbloom C.A. & Skinner R. 2001. Dietary practice, attitudes, and physiological status of collegiate freshman football players. *Journal of strength and conditioning*, 15:507-513.
- Kenney, W.L., Wilmore, J. & Costill, D. 2015. *Physiology of sport and exercise 6th Edition*. Human kinetics.

- Kerksick, C., Harvey, T., Stout, J., Campbell, B., Wilborn, C. & Kreider R. 2008. International Society of Sports Nutrition Position Stand: Nutrient timing. *Journal of International Society of Sports Nutrition*, 5:17.
- Kimball, S.R., Farrell, P.A. & Jefferson, L.S. 2002. Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *Journal of applied physiology*, 93(3):1168-1180.
- Kimiyiwe, M.E. & Simiyu, N.W.W. 2009. The extent of dietary supplements use by male rugby players in Kenya. *Journal of applied biosciences*, 22:1306-1311.
- Kirwan, R.B., Kordick, L.K., McFarland, D.L., Clark, K. & Miles, M.P. 2012. Dietary, Anthropometric, Blood lipid and Performance Patterns of American College Football Players during 8 weeks of training. *International journal of sport nutrition and exercise metabolism*, 22:444 -451.
- Koopman R., Pannemans D.L., Jeukendrup A.E., Gijsen A.P., Senden J.M., Halliday D., Saris W.H., van Loon L.J. & Wagenmakers A.J. 2004. Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. *American journal of physiology, endocrinology and metabolism*, 287:E712-720.
- Kraemer W.J., Hatfield D.L., Spiering B.A., Vingren J.L., Fragala M.S., Ho J.Y., Volek J.S., Anderson J.M. & Maresh C.M. 2007. Effects of a multi-nutrient supplement on exercise performance and hormonal responses to resistance exercise. *European journal of applied physiology*, 101:637-646.
- Kraemer, W.J., French, D.N., Spiering, B.A., Volek, J.S., Sharman, M.J., Ratamess, N.A. & Maresh, C.M. 2005. Cortisol supplementation reduces serum cortisol responses to physical stress. *Metabolism*, 54(5):657-668.
- Kreider, R.B., Wilborn, C.D., Taylor, L., Campbell, B., Almada, A.L., Collins, R., Cooke, M., Earnest, C.P., Greenwood, M., Douglas K.S., Kerksick, C.M., Kleiner, S.M., Leutholtz, B., Lopez, H., Lowery, L.M., Mendel, R., Smith, A., Spano, M., Wildman, R., Willoughby, D.S., Ziegenfuss, T.N. & Antonio, J. 2010. ISSN exercise & sport nutrition review: research & recommendations. *Journal of the International Society of Sports Nutrition*, 7(7):9-10.
- Kreider, R.B., Melton, C., Greenwood, M., Rasmussen, C., Lundberg J., Earnest, C. & Almada. A. 2003. Effects of oral D-ribose supplementation on anaerobic capacity and selected metabolic markers in healthy males. *International journal of sport nutrition, exercise and metabolism*, 13:76–86.
- Kreider, R.B. 1999. Dietary supplements and the promotion of muscle growth with resistance exercise. *Sports medicine*, 27(2):97-110.
- Krueger, R.A. & Casey, M.A. 2009. Focus groups: A practical guide for applied research. 4th ed. Thousand Oaks, CA: Sage.
- Lako, J.V., Sotheeswaran, S. & Christi, K.S. 2010. Food habits and nutritional status of Fiji rugby players. *World Academy of Science, Engineering and Technology*, 68:742-7.

- Leech, N.L. & Onwuegbuzie, A.J. 2009. A typology of mixed methods research designs. *Quality & quantity*, 43(2):265-275.
- Lemon, P.W.R. 1995. Do athletes need more dietary protein and amino acids? *International Journal of Sport Nutrition*, S39-S61.
- Lombard M.J., Steyn N.P., Charlton K.E. & Senekal M. Application and interpretation of multiple statistical test to evaluate validity of dietary intake assessment methods. *Nutrition journal*, 14:40.
- Lowery, L. & Forsythe, C.E. 2006. Protein and overtraining: potential applications for free-living athletes. *Journal of the International Society of Sports Nutrition*, 3(1):42-50.
- Luden, N.D., Saunders, M.J. & Todd, M.K. 2007. Postexercise carbohydrate-protein-antioxidant ingestion decreases plasma creatine kinase and muscle soreness. *International journal of sport nutrition and exercise metabolism*, 17(1):109.
- Lundy, B., O'Connor, H. & Pelly, F. 2006. Anthropometric characteristics and competition dietary intakes. *International journal of sport nutrition and exercise metabolism*, 16:199-213.
- Manore, M.M., Barr, S.I. & Butterfield, G.A. 2000. Nutrition and athletic performance. A joint position statement of the American Dietetic Association, the Dieticians of Canada, and the American College of Sports Medicine. *Medicine and science in sports and exercise*, 32:2130-2145.
- Maughan, R.J. Greenhaff, P.L. & Hespel, P. 2011. Dietary supplements for athletes: Emerging trends and recurring themes. *Journal of sports sciences*, 29 (1):57-66
- Miller, B.F. 2007. Human muscle protein synthesis after physical activity and feeding. *Exercise and sport sciences reviews*, 35(2):50-55.
- Millward, D.J. 2004. Macronutrient intakes as determinants of dietary protein and amino acid adequacy. *The journal of nutrition*, 134(6):1588S-1596S.
- Millward, D.J. & Jackson, A.A. 2004. Protein/energy ratios of current diets in developed and developing countries compared with a safe protein/energy ratio: implications for recommended protein and amino acid intakes. *Public health nutrition*, 7(03):387-405.
- Moore D.R., Robinson M.J. & Fry J.L. 2009. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American journal of clinical nutrition*, 89:161–168.
- Munteanu, A.M., Manuc, D., Caramoci, A., Vasilescu, M. & Ionescu, A. 2014. Nutrition timing in top athletes. *Medicina Sportiva: Journal of Romanian Sports Medicine Society*, 10(3):2357.
- Noakes, M., Keogh, J.B., Foster, P.R. & Clifton, P.M. 2005. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *The American journal of clinical nutrition*, 81(6):1298-1306.
- Nuttall F.Q., Gannon M.C. & Jordan K. 2004. The metabolic response to ingestion of proline with and without glucose. *Metabolism*, 53:241-246.

- Nuttall F.Q., Schweim K.J. & Gannon M.C. 2006. Effect of orally administered phenylalanine with and without glucose on insulin, glucagon and glucose concentrations. *Hormone and metabolic research*, 38:518-523.
- Petrie, H.J., Stover, E.A. & Horswill, C.A. 2004. Nutritional concerns for the child and adolescent competitor. *Nutrition*, 20(7): 620-631.
- Phillips, S.M. & Van Loon L.J. 2011. Dietary protein for athletes: from requirements to optimum adaptation. *Journal of sports science*, 29(1):29-38.
- Phillips, S. M. 2012. Dietary protein requirements and adaptive advantages in athletes. *British journal of nutrition*, 108(S2):S158-S167.
- Phillips, S.M. 2011. Dietary protein requirements and adaptive advantages in athletes. *British Journal of Nutrition*, 108:158-167.
- 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. 2004. Protein requirements and supplementation in strength sports. *Nutrition*, 20(7):689-695.
- Poortmans, J.R., Carpentier, A., Pereira-Lancha, L.O. & Lancha, A. 2012. Protein turnover, amino acid requirements and recommendations for athletes and active populations. *Brazilian journal of medical and biological research*, 45(10):875-890.
- Potgieter, S. 2013. Sport nutrition: A review of the latest guidelines for exercise and sport nutrition from the American College of Sport Nutrition, the International Olympic Committee and the International Society for Sport Nutrition. *South African Journal of Clinical Nutrition*, 26(1):11-13.
- Potgieter, S., Visser, J., Crowkamp, I., Markides, M., Nascimnto, J. & Scott, K. 2014. Body composition and habitual and match-day dietary intake of the FNB Maties Varsity Cup rugby players. *South African journal of sports medicine*, 26(2):35-43.
- Reitelseder, S., Agergaard, J., Doessing, S., Helmark, I.C., Lund, P., Kristensen, N.B. & Holm, L. 2011. Whey and casein labeled with L-[1-13C] leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *American journal of physiology-endocrinology and metabolism*, 300(1):E231-E242.
- Rasmussen B.B., Tipton K.D., Miller S.L., Wolf S.E. & Wolfe R.E. 2000. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *Journal of applied physiology*. 88:386-392
- Ratamess, N.A., Kraemer, W.J., Volek, J.S., Rubin, M.R., Gomez, A.L., French, D.N. & Dioguardi, F. 2003. The effects of amino acid supplementation on muscular performance during resistance training overreaching. *The journal of strength & conditioning research*, 17(2):250-258.

- Rennie, C.D., Brose, A.N., Carter, S.L. & Tarnopolsky, M.A. 2000. Muscle enzyme changes following endurance exercise in males and females. *Medicine and Science in Sports and Exercise*, 32(5):S263.
- Rennie, M.J. & Tipton, K.D. 2000. Protein and amino acid metabolism during and after exercise and the effects of nutrition. *Annual Review of Nutrition*, 20(1):457-483.
- Rodriguez N.R., DiMarco N.M. & Langley S. 2009. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the American Dietetic Association*, 109(3):509-527
- Rosenbloom, C.A., Jonnalagadda, S.S. & Skinner, R. 2002. Nutrition knowledge of collegiate athletes in a Division I National Collegiate Athletic Association institution. *Journal of the American Dietetic Association*, 102:418–420.
- Rowlands, D.S., Thorp, R.M., Rossler, K., Graham, D.F. & Rockell, M.J. 2007. Effect of protein-rich feeding on recovery after intense exercise. *International journal of sport nutrition and exercise metabolism*, 17(6):521.
- Rowlands, D.S., Rössler, K., Thorp, R.M., Graham, D.F., Timmons, B.W., Stannard, S.R. & Tarnopolsky, M.A. 2007. Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men. *Applied physiology, nutrition and metabolism*, 33(1):39-51.
- Saunders, M.J., Luden, N.D. & Herrick, J.E. 2007. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents post-exercise muscle damage. *The journal of strength & conditioning research*, 21(3):678-684.
- Saunders M.J., Kane M.D. & Todd M.K. 2004. Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Medicine and science in sports and exercise*, 36:1233-1238.
- Scofield D.E. & Unruh S. 2006. Dietary supplement use among adolescent athletes in central Nebraska and their sources of information. *Journal of strength and conditioning research*, 20(2):452-455.
- Schoenfeld, B.J., Aragon, A.A. & Krieger, J.W. 2013. The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *Journal of the International Society of Sports Nutrition*, 10:53.
- Schokman, C.P., Rutishauser, I.H. & Wallace, R.J. 1999. Pre-and postgame macronutrient intake of a group of elite Australian football players. *International journal of sport nutrition*, 9:60-69.
- Schröder, H., Navarro, E., Mora, J., Galiano, D. & Tramullas, A. 2001. Effects of α -tocopherol, β -carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *European journal of nutrition*, 40(4):178-184.
- Simiyu, N.W.W. 2010. Individual and institutional challenges facing student athletes on US college campuses. *Journal of physical education and sport management*, 1(2):16-24

- Slater G. & Phillips S.M. 2011. Nutrition guidelines for strength sports: sprinting, weightlifting, throwing events, and bodybuilding. *Journal of sports science*, 29(S1):S67-S77.
- Sobal, J. & Marquart, L.F. 1994. Vitamin/mineral supplement use among athletes: A review of literature. *International journal of sport nutrition*, 4:320-334.
- Smart, D.J., Gill, N.D., Beaven, C.M., Cook, C.J. & Blazevich, A.J. 2008. The relationship between changes in interstitial creatine kinase and game-related impacts in rugby union. *British journal of sports medicine*, 42(3):198-201.
- Stark, M., Lukaszuk, J., Prawitz, A. & Salacinski, A. 2012. Protein timing and its effects on muscular hypertrophy and strength in individuals engaged in weight-training. *Journal of the International Society of Sports Nutrition*, 9(1):54.
- Strachan, K. 2009. Observational descriptive study depicting current perceptions and usage practices of nutritional supplements amongst adolescent rugby-playing school boys from the Kwazulu Natal region. PhD dissertation, University of Stellenbosch.
- Tang, J.E., Moore, D.R., Kujbida, G.W., Tarnopolsky, M.A. & Phillips, S.M. 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of applied physiology*, 107(3):987-992.
- Teddlie, C. & Tashakkori, A. 2009. Foundations of Mixed Methods Research. *International journal of multiple research approaches*, 4:73-88.
- Tipton, K.D. & Stuart M.P. 2013. Dietary protein for muscle hypertrophy. *Health and exercise science research*, 73-84.
- Tipton, K.D., Rasmussen, B.B., Miller, S.L., Wolf, S.E., Owens-Stovall, S.K., Petrini, B.E. & Wolfe, R.R. 2011. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *American journal of physiology, endocrinology & metabolism*, 281:197-206.
- Tipton, K.D. 2010. Nutrition for acute exercise-induced injuries. *Annals of nutrition and metabolism*, 57(2):43-53.
- Tipton, K. & Ferrando, A. 2008. Improving muscle mass: response of muscle metabolism to exercise, nutrition and anabolic agents. *Essays in biochemistry*, 44:85-98
- Tipton K.D. & Witard O.C. 2007. Protein requirements and recommendations for athletes: relevance of ivory tower arguments for practical recommendations. *Clinical sports medicine*, 26:17-36.
- Tipton K.D., Elliott T.A., Cree M.G., Wolf S.E., Sanford A.P. & Wolfe R.R. 2004. Ingestion of casein and whey proteins results in muscle anabolism after resistance exercise. *Medicine and science in sports and exercise*, 36:2073-2081.
- Tipton, K.D. & Wolfe, R.R. 2004. Protein and amino acids for athletes. *Journal of sports sciences*, 22(1):65-79.

- Tipton K.D., Rasmussen B.B., Miller S.L., Wolf S.E., Owens-Stovall S.K., Petrini B.E. & Wolfe R.R. 2001. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *American journal of physiology, endocrinology and metabolism*, 281:E197-206.
- Tipton K.D., Ferrando A.A., Phillips S.M., Doyle D. & Wolfe R.R. 1999. Post-exercise net protein synthesis in human muscle from orally administered amino acids. *American journal of physiology* 276:E628–E634.
- Tarnopolsky, M. 2004. Protein requirements for endurance athletes. *Nutrition*, 20(7):662-668.
- Tarnopolsky, M., Parise, G., Fu, M.H., Brose, A., Parshad, A., Speer, O. & Wallimann, T. 2003. Acute and moderate-term creatine monohydrate supplementation does not affect creatine transporter mRNA or protein content in either young or elderly humans. In *Guanidino Compounds in biology and medicine*, 159-166.
- Tomé, D. & Bos, C. 2000. Dietary protein and nitrogen utilization. *The journal of nutrition*, 130(7):1868S-1873S.
- Volek, J.S., Sharman, M.J. & Forsythe, C.E. 2005. Modification of lipoproteins by very low-carbohydrate diets. *The journal of nutrition*, 135(6):1339-1342.
- Volek, J.S., VanHeest, J.L. & Forsythe, C.E. 2005. Diet and exercise for weight loss. *Sports medicine*, 35(1):1-9.
- Volek, J.S., Sharman, M.J., Gómez, A.L., DiPasquale, C., Roti, M., Pumerantz, A. & Kraemer, W.J. 2004. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *Journal of the American College of Nutrition*, 23(2):177-184.
- Volek, J.S., Sharman, M.J., Love, D.M., Avery, N.G., Scheett, T.P. & Kraemer, W.J. 2002. Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism*, 51(7):864-870.
- Walsh, M., Cartwright, L., Corish, C., Sugrue, S. & Wood-Martin, R. 2011. The body composition, nutritional knowledge, attitudes, behaviours, and future education needs of senior schoolboy rugby players in Ireland. *International journal of sport nutrition and exercise metabolism*, 21 (5):365-376
- Witard, O.C., Jackman, S.R., Breen, L., Smith, K., Selby, A. & Tipton, K.D. 2014. Myofibrillar muscle protein synthesis rates subsequent meal in response to increasing doses of whey protein at rest and after resistance exercise. *The American journal of clinical nutrition*, 99:86-95.
- White J.P., Wilson J.M., Austin K.G., Greer B.K., St John N. & Panton L.B. 2008. Effect of carbohydrate-protein supplement timing on acute exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 5:5.
- Wilborn, C.D., Taylor, L.W., Outlaw, J., Williams, L., Campbell, B., Foster, C.A. & Hayward, S. 2013. The effects of pre-and post-exercise whey vs. casein protein consumption on body composition and performance measures in collegiate female athletes. *Journal of sports science & medicine*, 12(1):74.

Willoughby D.S., Stout J.S. & Wilborn C.D. 2007. Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass and strength. *Amino acids*, 32:467-477.

Wilkinson, S.B., Tarnopolsky, M.A., MacDonald, M.J., MacDonald, J.R., Armstrong, D. & Phillips, S.M. 2007. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *The American journal of clinical nutrition*, 85(4):1031-1040.

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.

WHO Technical Report Series 935. Protein and Amino Acid Requirements in Human Nutrition: report of a joint FAO/ WHO/UNU expert consultation. Report of a JointWHO/ FAO/UNU Expert Consultation. 2011.

Wolfe, R. R. 2000. Protein supplements and exercise. *The American Journal of Clinical Nutrition*, 72(2):551s-557s.

Zieve, D. 2009. In Protein in diet: MedlinePlus Medical Encyclopedia. Retrieved June 1, 2010, from <http://www.nlm.nih.gov/medlineplus/ency/article/002467.htm>

Zinn, C., Schofield, G. & Wall, C. 2006. Evaluation of sports nutrition knowledge of New Zealand premier club rugby coaches. *International journal of sport nutrition and exercise metabolism*, 16(2):214.

ANNEXURE A: ETHICAL APPROVAL FORM

Private Bag X8001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

**Institutional Research Ethics Regulatory
Committee**

Tel +27 18 299 4849
Email Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by Health Research Ethics Committee, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: KNOWLEDGE AND PERCEPTIONS OF NORTH-WEST UNIVERSITY RUGBY PLAYERS ON TIMING OF PROTEIN INGESTION	
Project Leader: Dr L Havemann-Nel	
Ethics number:	N W U - 0 0 0 2 2 - 1 5 - A 1
	<small>institution Project Number Year Status</small>
	<small>Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation</small>
Approval date: 2015-07-24	Expiry date: 2016-05-01 Category N/A

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC:
 - annually (or as otherwise requested) on the progress of the project,
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-IRERC. Would there be deviations from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-IRERC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,
 - the required annual report and reporting of adverse events was not done timely and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis
Digitally signed by Linda du Plessis
 DN: cn=Linda du Plessis, o=NWU,
 ou=Vaal Triangle Campus,
 email=Linda.duplessis@nwu.ac.za,
 c=ZA
 Date: 2015.07.27 10:56:27 +0200

Prof Linda du Plessis
 Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

**ANNEXURE B: WRITTEN INFORMED CONSENT FORM RELIABILITY
STUDY**

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR:

A study to test the reliability of a knowledge questionnaire on nutrition

REFERENCE NUMBER: NWU-00022-15-S1

PRINCIPAL INVESTIGATOR: Dr Lize Havemann-Nel

ADDRESS:

Building G16 (Room 148)

11 Hoffman Street

North-West University

Potchefstroom

CONTACT NUMBER: 018 299 2399

You are being invited to take part in a study to validate a nutrition knowledge questionnaire for a research project that forms part of a MSc. degree in Nutrition at the North-West University in Potchefstroom. The purpose of this study is to test the questionnaire for reliability. Please take some time to read the information presented here, which will explain the details of this study. Please ask the researcher any questions about any part of this study that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you will be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU NWU-00022-15-S1) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this study all about?

This study will be conducted in lecture room 167 in building G16 at the North-West University, Potchefstroom campus and will involve the completion of a short multiple choice nutrition questionnaire that will be handed out by the researcher. The study will be conducted to test if the questionnaire that we have developed to assess nutrition knowledge amongst university athletes is reliable. Reliability is the extent to which a test (questionnaire) produces the same results during repeated trials. Therefore you will be required to report to room 167 in building G16 at the North-West University at two separate occasions 15 days apart to complete a questionnaire.

The objective of the study is to:

- Assess the reliability of a nutrition knowledge questionnaire.

Why have you been invited to participate?

- You have been invited to participate in the study because you are either a North-West University hockey player or a North-West University cricket player.
- You will be excluded if you are also a North-West University rugby player.

What will your responsibilities be?

An appropriate date and time will be arranged with your coach. On this appointed date and time you will be expected to report to lecture room 167 in building G16 on Potchefstroom campus. On arrival you will be welcomed and the procedures of the study will again be explained. You will be required to complete a short knowledge questionnaire (12 multiple choice questions) on nutrition. The lecture room in building G16 is equipped with tables and chairs where you will be seated at your own table to comfortably complete the questionnaire. The researcher will provide you with a pen to complete the knowledge questionnaire. You will also receive a sticker with your participant number. You will be asked to write your participant number on the knowledge questionnaire that will be handed out to you, and to complete the questionnaire as best as you can, on your own without help from a friend. Completing the questionnaire will not take you longer than 15-20 minutes, after which the questionnaires will be handed in. Fifteen days after completing the questionnaire for the first time, you will be required to return to lecture room 167 again to complete a second questionnaire on nutrition. Procedures on this day will follow according to your first visit.

Will you benefit from taking part in this research?

You will not receive any financial payments or incentives. You will receive the outcome of your knowledge questionnaire together with the correct answers to all the questions once all the data has been analysed. You will also receive a short summary of the study results on request.

Are there risks involved in your taking part in this research?

Questionnaires will be completed in a room where you can be comfortably seated with enough space and time to complete the questionnaire. The questionnaire will be completed in silence and no group discussion or group work will be allowed during the completion of the questionnaire. No one except for the researchers involved will know what you have answered, and only your participant number will be written on the questionnaire to ensure confidentiality. The questionnaire will be in English but if you rather want an Afrikaans questionnaire, one will be provided for you.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. If you experience any discomfort of any kind during the completion of the questionnaire, you are allowed to leave the lecture room at any point and your questionnaire will be shredded and your data will not be used for this study.

Who will have access to the data?

Only the researchers involved with this study will have access to the data. Every effort will be made to ensure the confidentiality of any identifying information that is obtained in connection with this study. All questionnaires will be anonymous, with only a participant number as identification.

What will happen with the data/questionnaires?

All the documents will be kept in a locked filing cabinet, and only the researchers involved in the study will have access to the data. After five years, all the documents from this study will be shredded.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study. There will also be no incentive if you do take part. Other than travelling to building G16 on Potchefstroom Campus at your own cost, and the time

that you dedicate to participate in this study, there will be no additional costs involved from your side to participate in the study.

Is there anything else that you should know or do?

You can contact Dr Lize Havemann-Nel at 018 299 2399, Lianri Swanepoel at 072 315 8790 or Dr Chrisna Botha-Ravyse at (016) 910-3368 if you have any further queries or encounter any problems.

You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at (018) 299 2089; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

The findings of the research will be shared with you after data are analysed and your level of knowledge will be reported.

Declaration by participant

By signing below, I agree to take part in a research **study aimed to test the reliability of a knowledge questionnaire on nutrition**

I declare that:

I have read this information and consent form and it is written in a language with which I am fluent and comfortable.

I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.

I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 20....

.....

.....

Signature of participant

Signature of witness

Declaration by person obtaining consent

I (*name*) declare that:

I explained the information in this document to
I encouraged him to ask questions and took adequate time to answer them.
I am satisfied that he adequately understands all aspects of the research, as discussed above
I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....

Signature of person obtaining consent

Signature of witness

Declaration by researcher

I (*name*) declare that:

I explained the information in this document to
I encouraged him/her to ask questions and took adequate time to answer them.
I am satisfied that he/she adequately understands all aspects of the research, as discussed above
I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....

Signature of researcher

Signature of witness

**ANNEXURE C: WRITTEN INFORMED CONSENT FORM CROSS
SECTIONAL STUDY**

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR:

Knowledge and perceptions of university rugby players on nutrition

REFERENCE NUMBER: NWU-00022-15-S1

PRINCIPAL INVESTIGATOR: Dr Lize Havemann-Nel

ADDRESS:

Building G16 (Room 148)
11 Hoffman Street
North-West University
Potchefstroom

CONTACT NUMBER: 018 299 2399

You are being invited to take part in a research project that forms part of a MSc. degree in Nutrition at the North-West University in Potchefstroom. The purpose of this research is to capture a deeper understanding of the knowledge and perceptions of North-West University rugby players on nutrition. Please take some time to read the information presented here, which will explain the details of this study. Please ask the researcher any questions about any part of this study that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you will be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00022-15-S1) and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be conducted in The FNB *High Performance Institute of Sport (HPI)* on the Potchefstroom Campus of the *North-West University* in room 229 on the 31st of August, 1st, 2nd and 3rd of September at 12:00 - 14:00. It will involve the completion of a nutrition knowledge questionnaire and anthropometric measurements, including weight and height. You may also be randomly selected to participate in a focus group discussion on nutrition perceptions (only 8 randomly selected participants from each team will take part in the focus group discussion). Experienced health researchers will collect the data. Anthropometric measures will be performed by an ISAK level 1 qualified anthropometrist. The knowledge questionnaire will be administered by the researcher and the focus group discussion will be conducted by researchers with expertise and experience in conducting focus group discussions.

The objectives of the study are to:

1. Determine the knowledge of university rugby players on nutrition
2. Examine the perceptions of university rugby players on nutrition

Why have you been invited to participate?

- You have been invited to participate because you are a NWU Rugby Institute rugby player. You have also meet the following inclusion criteria: Playing for the 1st, 2nd, 3rd, u21 A or U/19A teams. You are between the ages of 18 and 24, currently enrolled in a course at the NWU and volunteering to participate.
- You will be excluded if: You are not part of the NWU rugby institute, not playing for one of the above mentioned teams, not enrolled in a course at the NWU, not between the ages of 18 and 24 and not agreeing to participate. You will also be excluded if you are registered for any of the following modules presented by a researcher on the study team (e.g. VOED323, NUTC321, VOED681, VOED876, NUTT322, NUTT311, NUTB311, or VNDL311).

What will your responsibilities be?

If you volunteer to participate in this study, we would ask you to do the following things:

On the appropriate date and time arranged with your coach, you will be expected to report to the HPI, room 229, with the rest of your team members who volunteered to participate in the study. On arrival you will be welcomed by the researcher, where the procedure of the study will again be explained. There will be three testing stations of which two stations will be visited by all participants. The duration of data collection at these two stations will not be longer than 30 minutes. The first station is the anthropometric station where your height and weight will be measured at least two times. You will be instructed to remove your shoes, heavy clothing and accessories (10 minutes per participant).

At the second station you will be expected to complete a short multiple-choice questionnaire taking about 15-20 minutes. The questionnaire will test your knowledge on nutrition. The questionnaire will be in English but an Afrikaans version will be available on request.

The third and last station will be the focus group discussion. Only eight participants from each team will be randomly selected to take part in the focus group discussion on nutrition that will take approximately 45 – 60 minutes. You will be seated on chairs that are placed in a circle in the designated quiet room, greeted and thanked for agreeing to participate in a focus group discussion on nutrition perceptions. You will also be encouraged to keep the focus group discussion confidential. The focus group discussion will be voice recorded and you will also be required to give verbal consent on the voice recorder, agreeing that only partial confidentiality can be ensured due to the fact that group members may disclose what was discussed during the focus group discussion. You will also receive an alias to protect your identity during transcription of the focus group discussion. The alias will be written on a name card and attached to your shirt where it is visible to the researcher and the other participants. The focus group discussion will be conducted in English, but you are allowed to ask for Afrikaans translation at any point during the focus group discussion. You are also allowed to answer in Afrikaans. During this discussion, only the researchers acting as interviewer and scribe/note taker will be present.

Will you benefit from taking part in this research?

You will not receive any financial payments or incentives. You will receive your anthropometry results (i.e. height, weight and BMI). You will receive the outcome of your knowledge questionnaire together with a memo of the questionnaire once all the data has been analysed. You will also receive a hand-out with practical nutritional recommendations on nutrition. Additionally you will receive a short summary of the study results on request.

Are there risks involved in your taking part in this research?

You may experience possible discomfort during the anthropometric measurements. However measuring weight and height are standard procedures in research studies and you should be well acquainted with these procedures since monitoring your weight is part of the sport of rugby. To minimize any discomfort, anthropometric measures will be performed professionally in an enclosed private room, where you will be required only to remove your shoes and heavy clothing or accessories. Apart from the researcher measuring your weight and height, you will be the only other person in the room.

Questionnaires will be completed in a room where you can be comfortably seated with enough space and time to complete the questionnaire. The questionnaire will be completed in silence and no group discussion or group work will be allowed during the completion of the

questionnaire. No one will know what you answered and only your participant number will be displayed on the questionnaire. The questionnaire will be in English but if you would like an Afrikaans questionnaire, one will be provided for you.

Focus group discussions pose the risk that confidentiality could be compromised. Total confidentiality cannot be guaranteed and only partial confidentiality can be ensured as group members may disclose what was discussed during the focus group discussions, outside the research setting. However, everyone taking part in the focus group discussion will be encouraged to keep the discussion confidential, even though the topic of discussion is not of a sensitive nature. You will need to give verbal consent on a voice recorder before the focus group discussion starts. In addition, you will be provided with an alias to protect your identity during the recording and transcription of the focus group discussion. Only you and seven other players from your team, the interviewer and the scribe (both more senior researchers on the research team with expertise in conducting focus group discussions) will be allowed in the focus group discussion room. No coaches, conditioning trainers, players from other teams or participants from the same team that was not randomly selected to participate in the focus group discussion will be allowed in the room during the focus group discussion. To minimize discomfort, focus group discussions will be performed in a room that is private and comfortable. You will only be addressed by your alias and only the alias will be used when the student researcher on the research team transcribe the data from the focus group discussions.

Water will be available at all times to ensure the comfort of the participants.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may ask to have the discussion or parts of it where you participated, removed from the study data. You may also refuse to answer any questions you don't feel comfortable answering, and still remain in the study. If you decide after the focus group discussion is finished that you don't want us to include your interview in the study, you can contact Dr. Lize Havemann-Nel and ask her to delete your part of the focus group discussion on the tape or transcript. Should you have the need for further discussions after the focus group or anthropometric measurements, an opportunity will be arranged for you to discuss your discomfort with the researchers.

Who will have access to the data?

Only the researchers involved in the study will have access to the data. Every effort will be made to ensure the confidentiality of any identifying information that is obtained in connection with this study. All questionnaires will be anonymous, with only a number as indication of

participant participation. Each audio-recorded interview will start with an opening statement of the date, time and a participant number or alias (used in place of your name). You will also receive a name tag with your alias written on it and you will be addressed by the interviewer on the alias. Should your name come up at any time during the focus group discussion, it will later be transcribed as your participant number or alias. There will be no way of identifying you from the transcribed focus group discussion. After the audiotape has been transcribed, the tape will be erased. All the transcribed documents will be kept in a locked filing cabinet, and only the researchers involved in the study will have access to them. After five years, all the documents from this study will be shredded and the hard drive containing electronic data will be deleted.

What will happen with the data/questionnaires?

All the documents will be kept in a locked filing cabinet, and only the researchers involved in the study will have access to the data. After five years, all the documents from this study will be shredded.

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study. There will also be no incentive if you do take part. Other than travelling to the HPI on Potchefstroom Campus at your own cost, and the time that you dedicate to participate in this study, there will be no additional costs involved from your side to participate in the study.

Is there anything else that you should know or do?

You can contact Dr Lize Havemann-Nel at 018 299 2399, Lianri Swanepoel at 072 315 8790 or Dr Chrisna Botha-Ravyse at (016) 910-3368 if you have any further queries or encounter any problems.

You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at (018) 299 2089; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

The findings of the research will be shared with you after data are analysed and your level of knowledge will be reported.

Declaration by participant

By signing below, I agree to take part in a research study on knowledge and perceptions of university rugby players on nutrition.

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 20....

.....

Signature of participant

.....

Signature of witness

Declaration by person obtaining consent

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him to ask questions and took adequate time to answer them.
- I am satisfied that he adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....

Signature of person obtaining consent

.....

Signature of witness

Declaration by researcher

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him to ask questions and took adequate time to answer them.
- I am satisfied that he adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....

Signature of researcher

.....

Signature of witness

ANNEXURE D: KNOWLEDGE QUESTIONNAIRE

Participant number: _____
Date: _____
Age: _____ years
Race: _____
Team: _____
Position of play: _____
Highest team played for: _____
Enrolled degree at NWU: _____

Protein timing knowledge questionnaire

Please answer the following questions by circling the correct letter. Each question only has **ONE** correct answer.

1. In a sport nutrition context, which one of the following statements explains ‘the timing of protein intake’ the best?

- A. The total duration it takes to consume protein throughout the day
- B. Timing oneself while consuming a protein rich source to promote muscle gain
- C. Planning your intake of protein sources during and around training in order to enhance performance benefits
- D. Consuming protein in a certain order throughout the day to promote muscle gain
- E. A & B

2. Which of the following athletes can benefit from optimal timing of protein ingestion?

- A. Strength and power athletes
- B. Team sport athletes
- C. Endurance athletes
- D. All of the above
- E. A & B

3. Which one of the following is not a potential benefit of eating protein at the right time in context to your training session:

- A. Increased muscle mass
- B. Optimal muscle protein synthesis after training
- C. Increased muscle glycogen storage
- D. Improved performance
- E. None of the above

4. The recommended amount of protein to consume *before* resistance exercise in combination with carbohydrate for an 80kg athlete is:

- A. 80 gram
- B. 12 – 20 gram
- C. 40 – 60 gram
- D. 100 – 120 gram
- E. 160 gram

5. Protein ingested *before* resistance training can potentially:

- A. Increase muscle gain during the training session
- B. Increase muscle protein synthesis in response to resistance training
- C. Provide the primary source of energy during the training session
- D. Improve strength during training
- E. B & D

6. What is the recommended ratio of carbohydrate-to-protein to consume *during* exercise?

- A. 3-4:1
- B. 1:1
- C. 1: 3-4
- D. 2:1
- E. 1:2

7. Protein consumption *during* exercise can potentially:

- A. Reduce endurance performance
- B. Increase glycogen stores
- C. Reduce muscle damage
- D. Promote training adaptations after resistance exercise
- E. B, C & D

8. The role of protein consumed *during* resistance exercise is primarily:

- A. To provide amino acids for increased muscle protein synthesis
- B. To provide energy for increased muscle protein synthesis
- C. To reduce muscle damage after exercise
- D. To reduce muscle protein synthesis during exercise
- E. A & C

9. The recommended amount of protein to consume *after* resistance exercise is:

- A. 50 – 65 gram (i.e. hand size fillet steak weighing 200 – 275 gram)
- B. 15 – 20 gram (i.e. 1 small-to-medium chicken breast)
- C. 20 – 25 gram (i.e. 1 medium-to-large fish fillet)
- D. 5 – 10 gram (i.e. 1 – 2 small eggs)
- E. More than 65 gram (1 large fillet steak weighing more than 275 gram)

10. The best time to consume protein *after* exercise or an event is?

- A. Approximately 30 minutes after exercise
- B. 3 hours after training
- C. 6 hours after training
- D. Before going to bed that night
- E. The next morning

11. Which one of the following protein sources has the greatest effect on muscle protein synthesis when consumed *after* resistance exercise?

- A. Milk
- B. Egg
- C. Meat
- D. Soya
- E. Chicken

12. Protein consumption in combination with carbohydrates *after* exercise is important to:

- A. Promote muscle protein synthesis
- B. Potentially improve muscle glycogen storage
- C. Reduce muscle gain
- D. Optimize post-exercise recovery
- E. A, B & D

**ANNEXURE E: PRE-DRAFTED FOCUS GROUP DISCUSSION
QUESTIONS**

Guiding questions for focus group discussion

1. What thoughts go through your head when you hear the words timing of protein intake?

2. When did you first hear of protein timing/timing of protein intake? (prompts: do you remember how old you were?)

3. Where did you hear about the term?

4. What do you consider to be timing of protein intake?

5. Do you think it is important to eat protein at the right time?

6. Do you think timing of protein intake is relevant/important/beneficial?

7. Relevant/beneficial/important for what?/why?/in what way? (Prompts: Physique? Strength? Rugby performance? Weight loss?)

8. What would you consider to be the best time to take/eat protein?

9. Why do you consider this to be the best time? (prompts: Did someone suggested that? Who? Did you read it somewhere?)

11. Do you ever make use of any of these protein timing strategies? If so when did you do this? (during training or competition)

12. Why do you make use of these strategies?

ANNEXURE F: ARTICLE 1 – AUTHOR INSTRUCTIONS

AUTHOR INSTRUCTIONS – The journal of Nutrition Education and Behaviour

The *Journal of Nutrition Education and Behaviour*, the official journal of the Society for Nutrition Education and Behaviour, is a refereed, scientific periodical that serves as a resource for all professionals with an interest in nutrition education and dietary and physical activity behaviours. The purpose of *JNEB* is to document and disseminate original research, emerging issues, and practices relevant to nutrition education and behaviour worldwide. The journal welcomes evidence-based manuscripts that provide new insights and useful findings related to nutrition education research, practice, and policy. The content areas of *JNEB* reflect the diverse interests of health, nutrition and education.

The *Journal of Nutrition Education and Behaviour* follows the guidelines for authorship from the International Committee for Medical Journal Editors. As such, the journal recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.
 2. Drafting the work or revising it critically for important intellectual content.
 3. Final approval of the version to be published.
 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- To satisfy the requirement for authorship, each contributor must meet all 4 criteria above. Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see <http://www.elsevier.com/copyright>).

Content list

Manuscript Preparation

1. General guidelines

Manuscripts (including the main text, references, and figure legends) should be saved without a title page as a single file, should be prepared in a 12-point typeface, double-spaced, and in a single column with 1-inch margins throughout. Keep the layout of the text as simple as possible. Do not justify text or hyphenate words. Use the software's bold, italic, subscript, and superscript functions. Use the software's table function to create tables, using rows and columns, not tabs and spaces, to align data.

Beginning with the Introduction, each manuscript page is numbered in the upper right-hand corner and each line of text is numbered consecutively. First-level headings are centred on the page, typed in uppercase, bolded letters, and followed by two blank lines. Second-level headings begin flush with the left margin, have each word capitalized and bolded, and are followed by one blank line. Third-level headings begin flush with the left margin, are written sentence style with a period at the end, and are bolded. Text follows immediately on the same line

Page and word limits exclude the abstract but include all other text, acknowledgments, tables, figures, and references. Manuscripts must not exceed word count limits or page limits.

Research Article: 20 double-spaced pages ($\leq 4,500$ words)

A structured abstract of 200 words or less organizes information with descriptive headings that begin flush with the left margin. Incomplete sentences are acceptable in a structured abstract for the sake of brevity. To facilitate selective electronic searches, structured abstracts include the following subheadings bolded and presented in the following order: Objective, Design, Setting, Participants, Intervention, Main Outcome Measure, Analysis, Results, Conclusions and Implications

Research Articles include the following major sections: Introduction, Methods, Results, Discussion and Implications for Research and Practice.

2. Style guidelines

JNEB adheres to the style recommendations outlined in the *American Medical Association Manual of Style*, 10th edition (<http://www.amamanualofstyle.com/>). Manuscripts should be written in good scientific English (American or British usage is accepted, but not a mixture of these).

Please note the following style requirements and format manuscripts accordingly before submission:

- Abbreviations and acronyms should be spelled out on first use, with the shortened versions immediately following in parentheses. Manuscripts should be limited to a total of five acronyms and abbreviations.
- “N” and “n” should be used as follows: “N” indicates a whole population or an epidemiological study; “n” indicates a sample or subpopulation.
- Sentences in unstructured abstracts or in the body of a manuscript may not begin with a numeral. Sentences in structured abstracts may begin with a numeral.
- Decimals should be used only to 1 degree more than the unit of measurement. For whole numbers, decimals need to be rounded to tenths; if precision of measurement is in the tenths, you may use hundredths (eg, with weight measured to the tenth of a pound, means may be expressed as hundredths). Most software will express results greater than the precision, it is not appropriate to use these figures in tables (eg, 34.1 mg niacin). The exception to this is percentages concerning people. For fewer than 100 people, please round to the nearest whole percentage, eg, 95% of participants (n = 80), rather than 95.3% of participants (n = 80).
- When reporting *P*, values less than .001 should be reported as such: $P < .001$. Actual values for *P* should be expressed to 2 digits for $P \leq .01$, whether or not *P* is significant. If *P* is $< .01$, there should be 3 significant figures reported (eg, $P = .008$). Use *P* values rather than alpha values. For general statistical guidelines, please read Guidelines for Statistical Methods for *JNEB*.

Quantitative and Qualitative Research

Key words: All structured and unstructured abstracts are accompanied by a list of 3 to 5 key words for indexing. Key words are selected from the listing of Medical Subject Headings (MeSH) outlined by MEDLINE. To maximize the likelihood that your paper will be identified appropriately by other researchers, educators, and administrators.

Tables: For submission, each table should be saved and uploaded as a separate file. Number tables consecutively in accordance with their appearance in the text. If there is only one table, then no number is assigned (eg, “Table”). Format tables as follows:

Title: Provide a table number and a descriptive title. Words in the title are capitalized. The title should describe the type of data included and give the sample size (n) unless it varies by measure/variable (in which case, n should be included within the table content).

Content: Not all data included in tables needs to be reported within the text of the manuscript. The most important results should be included in the text, but repeating results that will not be discussed further is discouraged. Bullets should not be used within a table. For qualitative tables, indentation of text may also be used within a section.

Footnotes: The order of items within the footnote is as follows: abbreviations, then statistical significance, then statistical test used. Any abbreviation used in the table should be spelled out in the footnote. If not included in the table content, statistical significance should be identified with an asterisk (eg, $*P < .05$; $P < .01$; $P < .001$; or *Significance based on 95% CI). Statistical test used (eg, chi-square, logistic regression) and statistical adjustments made to models should also be identified.

Statistics: Report means and standard deviations if the data have a normal distribution; report the interquartile range (IQR) and the median if the data are not normally distributed. Standard error of the mean (SEM) should only be used if multiple samples are gathered (eg, groups of schools). Confidence intervals (CIs) should be included if relative risk or odds ratios are given in the table. The statistical significance (*P*) may be included as the number (eg, $P < .05$) or indicated by an asterisk and footnote (see Footnotes section, above). Superscripted lowercase letters may be used if differences among several groups are to be shown. Differences between 2 or more groups should include a column for *P* or an asterisk to indicate significance, where appropriate. Refer to the “General style and form and writing style” section above for guidance on the number of decimal places or significant digits to show in tables.

Figures and artwork: For submission, each figure should be saved and uploaded as a separate file. Number figures consecutively in accordance with their appearance in the text. If there is only one figure, then no number is assigned (eg, “Figure”). Format figures as follows:

Caption: Figure captions should be presented at the end of the manuscript just after the references (captions should not be attached to the figures themselves). Captions constitute a distinct section of the manuscript and should start on a new page. Ensure that each illustration has a caption. A caption should consist of a brief title and a description of the illustration. Figure captions should be written in sentence format. Example of unacceptable caption: “Body Mass Index (BMI) versus calories.” Example of

acceptable caption: "Body Mass Index (BMI) versus calories consumed after 3-month intervention with 10- to 12-year-olds." Figure captions should also explain any abbreviations or statistical tests (eg, chi-square, logistic regression). Keep text in figures to a minimum; instead, use figure captions to explain all symbols and abbreviations used.

Content: Lettering and data symbols must be clear and consistent on each figure. Use uniform lettering and size your original artwork consistently. Only use the following fonts in illustrations: Arial, Courier, Helvetica, Times New Roman, and Symbol. Titles, explanations, and definitions of abbreviations must be noted in the legends, not on the figures themselves.

References: Each new reference introduced in the text is numbered sequentially. The reference number appears superscripted immediately following related text. The reference list is double-spaced and numbered to correspond with citations in text. Reference style follows the system described in the *American Medical Association Manual of Style*, 10th edition, except that issue numbers are not included in journal references. MEDLINE abbreviations are used for periodical titles. If a standard abbreviation is not available on MEDLINE, cite the full title. Examples of different reference types follow:

Journal Article: Olson CM. Tracking of food choices across the transition to motherhood. *J Nutr Educ Behav.* 2005;37:129-136.

Book: Glanz K, Rimer BK, Lewis FM, eds. *Health Behavior and Health Education: Theory, Research, and Practice.* 3rd ed. San Francisco, CA: Jossey-Bass Publishers; 2002.

Book Chapter: Baranowski T, Perry CL, Parcel GS. How individuals, environments, and health behavior interact. In: Glanz K, Rimer BK, Lewis FM, eds. *Health Behavior and Health Education: Theory, Research, and Practice.* 3rd ed. San Francisco, CA: Jossey-Bass Publishers; 2002:165-184.

Footnotes: Footnotes are not permitted except in tables. In tables, footnotes are superscripted; lowercase letters (or other common designators) are used to indicate significant differences within rows (see the "Tables" section, above).

ANNEXURE G: ARTICLE 2 – AUTHOR INSTRUCTIONS

AUTHOR INSTRUCTIONS – International Journal of Sports Nutrition and Exercise Metabolism (IJSNEM)

IJSNEM publishes a range of different types of papers, including original research investigations. The common goal is to promote new and high-impact insights into sport nutrition and exercise metabolism, as well as the application of the principles of biochemistry, physiology, and nutrition to sport and exercise. Original research with human subjects will be emphasized. Please see the separate author guidelines. Note that even when papers are commissioned, each will undergo peer review, and unless prior authorisation has been provided by the Editor in chief or Special Projects editor, all papers must conform to the submission guidelines.

Content list

Manuscript preparations

1. General guidelines

Language: All manuscripts must be written in English, with attention to concise language, a logical structure and flow of information, and correct grammatical style.

File type: All manuscripts should be submitted in Microsoft Word or another comparable word processing software program. Figure files can be submitted in other formats (see **Figures and tables** section below).

Format: Manuscripts should include the following elements in the order indicated: 1. Title page, 2. Abstract and keywords, 3. Text, 4. Acknowledgments, authorships, declarations of funding sources and conflicts of interest, 5. References, 6. Tables, 7. Figure legends, and 8. Figures. The title page should include names and addresses of all authors and full contact details for the corresponding author. Manuscripts should be double-spaced with wide margins and should include continuous line numbers in the text. Pages should be numbered in the upper right corner. Each Table and Figure should be presented on a separate page; headings should be included with each Table while figure legends should be aggregated on a separate page at the conclusion of references.

Title page: The manuscript must have a separate title page including title of article, name(s) of author(s), institutional affiliation(s), running head, and e-mail address and phone number of the author who is to receive the galley proofs. The title of the paper should be limited to 25 words. The running title (an abbreviated version of the title that is printed at the top of the page in the formatted journal version) should be limited to 8 words.

Abstract: The abstract should be a maximum of 250 words and be written in one continuous paragraph without subheadings. Abstracts should showcase the new information presented in the paper, either in the form of original research data or as a novel insight into an established issue. Abstracts reporting original research must include sufficient data to support any conclusion reached. It is not satisfactory to simply describe what was found (such as, "the treatment group improved more than the control group") nor to say simply that "the results will be discussed". References should not be included.

Keywords: three keywords or phrases not included in the manuscript title.

Acknowledgement of support: All funding sources and potential conflicts of interest should be declared at the end of the text.

Authorship guidelines: Only individuals who have made a substantial contribution to the manuscript, as described below, should be credited as co-authors. Each author should have participated sufficiently in the work to take public responsibility for the content. At the end of the text, the acknowledgements section of the paper should identify the role played by each author: Example for a paper featuring four authors: LMB, GC, NR, and BP. All authors approved the final version of the paper".

Use of human and animal subjects: *IJSNEM* requires that all submitted studies using human or animal subjects conform to the policies established by the U.S. Department of Health, Education, and Welfare and the American Physiological Society. Manuscripts should include a clear statement to the effect that studies had prior approval from a formally constituted ethics review board in the case of human studies

and that informed consent was obtained in writing from participants (or guardians for participants under the age of 18 years),

Methods in sport nutrition research: To assist with the design, implementation, and interpretation of studies in sport nutrition, *IJSNEM* has commenced the publication of a series of reviews on methodologies in sport nutrition research. These articles provide commentary from experts in a variety of fields on optimum ways to conduct and report studies on aspects of sport nutrition research. They can be downloaded from the *IJSNEM* Website [here](#).

Figures and tables: Figures should be professional in appearance and have clean, crisp lines. Hand drawings and hand lettering are not acceptable. Color is not permitted: figures should use black and white or gray shading only. Labels should be proportionate with the size of the figures on the journal page. Digital photos should be 300 dpi at full size, and digital line art should be 600 dpi. Figures can be submitted electronically in TIFF or PDF file formats.

When tabular material is necessary, it should not duplicate the text. Tables should be formatted using the table function of the word-processing program rather than by aligning columns in text with tabs and spaces or using text boxes. Tables should be double-spaced on separate sheets and each should include a brief title and a legend that highlights any statistically significant findings.

Statistical analysis: Papers submitted to the journal may be sent for review to a statistician if the Editor is not satisfied that appropriate procedures have been followed. When data sets are normally distributed, variance should be given as the *SD* rather than *SEM*. Nonparametric statistical analysis should be used when data sets are not normally distributed.

Reference style: *IJSNEM* follows a modified version of the style laid out in the *Publication Manual of the American Psychological Association* (APA), 6th ed. References should be listed in alphabetical order at the end of the text and should be cited in the text using author name(s) and date of publication. In the case of in-text citations, where there are more than two authors, the first author's name can be followed by "et al." Example: "Burke, Clooney, Pitt, and Riewoldt (2009) found that supplementation achieved positive outcomes" can be replaced by "Supplementation was found to achieve positive outcomes (Burke et al., 2009)." References should not be numbered in the reference list. Examples of the three most common forms of references are shown below. For other variations, please consult the APA manual.

Burke, L M., Clooney, G., Pitt, B., & Riewoldt, N. (2009). Cacao supplementation does not affect sprint performance in elite team sport players. *Journal of Clinical Inspiration*, 67, 1966–1971.

Wadler, G.I., & Hainline, B. (1989). *Drugs and the athlete*. Philadelphia: F.A. Davis.

Haymes, E. Proteins, vitamins, and iron. (1983). In M.H. Williams (Ed.), *Ergogenic aids in sport* (pp. 27–55). Champaign, IL: Human Kinetics.

Submission: Manuscripts should be submitted electronically via *IJSNEM*'s Manuscript Central site. The Manuscript Central system manages the electronic transfer of manuscripts throughout the article review process, providing step-by-step instructions and a user-friendly design.

The review process: Manuscripts are read by an editor and two reviewers; reviews will not be blind. Authors are required to provide the names and email addresses of a minimum of two potential peer reviewers when they submit their manuscripts. The review process should not take more than about 6-8 weeks.

Copyright: Authors of manuscripts accepted for publication are required to transfer copyright to Human Kinetics. Please visit Manuscript Central to download *IJSNEM*'s copyright form located under the Instructions & Forms link in the upper right corner. You do not need an account to access this information.

Specific guidelines are provided for the following papers:

Original Research

Scope: Original Research papers should cover topics of novelty and high impact in relation to sport nutrition or exercise metabolism. Even in cases where research has been conducted carefully and has

been appropriately written up, a manuscript may be rejected if it is deemed to be of insufficient interest and quality to attract attention.

Title: Where possible, the title should be brief but instructive of the outcome of the study: Example: “Caffeine fails to improve 200 m swimming time in elite swimmers” is preferred to “Effect of caffeine on swimming performance in elite swimmers”

Text: *Length.* 3,000 words (excludes title page, abstract, acknowledgements, references, figures, tables)

Sections. Sections include Introduction, Methods, Results, and Discussion; each of these sections should follow the standard processes. Where appropriate, the text should conclude with two brief sections: **novelty statement** (one or two sentences should sum up the new information that has been gained as a result of the study) and **practical application statement** (one or two sentences should sum up the way that this information could be put into practice).

Acknowledgements: Note that the contribution of each author to the paper should be outlined.

References: A maximum of 40 references can be cited.

Figures and tables: A total of six figures and/or tables may be used to illustrate the data in this study. The total of six assumes no more than one page for each figure. If a figure has multiple panels requiring more than one page, the total number of figures should be reduced accordingly. If you feel that additional panels or figures are needed, please be sure to address this in your cover letter.

ANNEXURE H: HANDOUT

Before and during training or games



For potential added benefits: experiment with adding protein to your pre-game meal

- Moderate amounts of protein in a pre-game meal
- Protein : 0.15 – 0.25g/kg bodyweight +
- Carbohydrates : 1 - 2g/kg bodyweight



- Protein can be added to carbohydrates during training/a game
- Carbohydrate:Protein ratio of 3-4:1 (3-4 times as much carbohydrates as protein)

Post workout smoothy



Berry nana-nuts

- 1 large banana
- 1 cup strawberries
- 1 cup low fat milk
- 1 tablespoon peanut butter
- 3 tablespoons all bran cereal
- 1/2 scoop strawberry whey protein

Peanut butter is packed with protein and healthy fats that stimulates muscle growth



Guidelines to protein timing



After training or games

- Example: when it is not possible to eat a meal **after training**
- Here's some handy snacks:



- After training (within 30-60min.):
- ↑Stimulation of muscle protein synthesis
 - ↑Muscle damage recovery/repair
 - ↑Maximum strength (long term)

Most important time to consume protein mainly for recovery



Within 30- 60minutes after training or a game .

- Amount : Resistance training: 1.3 - 1.8g/kg bodyweight
Endurance exercise: 1.2 - 1.7g/kg bodyweight
- Composition: 65—85g high GI carbohydrates +
20—25g high quality protein
- Source: Essential Amino Acids (EAA) like low fat milk.



- Example: If it is possible to eat a meal **after training**



- After training (within 30-60min.):
- ↑Body composition (long term)
 - ↑ Muscle glycogen stores
 - * Possible indirect performance benefits