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PROTEIN SUPPLEMENTS AND THEIR EFFECT ON PHYSICAL FITNESS QUALITIES OF MEN HANDBALL TEAM PLAYERS IN THE CASE OF DEBRE MARKOS UNIVERSITY

FRIEW, AMARE

BAHIR DAR UNIVERSITY
SPORT ACADEMY

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BY:
FRIEW AMARE

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A THESIS SUBMITTED TO BAHIR DAR UNIVERSITY, SPORT ACADEMY, IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE DEGREE OFMASTERS IN COACHING HANDBALL

BY: FRIEW AMARE

ADVISOR: BEWKETU CHEKOL (ASS. Pro.)

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BAHIR DAR

BAHIR DAR UNIVERSITY

SPORT ACADEMIY POST GRADUATE PROGRAM

GRADUATE STUDIES APPROVAL PAGE

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I hereby certify that I have supervised, read, and evaluated this thesis/dissertation titled "Protein supplements and their effect on physical fitness qualities of men handball team players in the case of Debre Markos University" by Mr. Friew Amare prepared under my guidance. I recommend the thesis be submitted for oral defence.

_____	_____	_____
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We hereby certify that we have examined this thesis entitled “Protein supplements and their effect on physical fitness qualities of men handball team players in the case of Debre Markos University” by Mr. Friew Amare. We recommend that Mr Friew Amare is approved for the degree of “Handball Coaching”.

Board of Examiners

_____	_____	_____
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_____	_____	_____
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DECLARATION OF AUTHORSHIP

I hereby that, this thesis is used for the partial fulfillment of the requirement for the Degree of Master of Science in Coaching handball on the title of "Protein supplements and their effect on physical fitness qualities of men handball team players in the case of Debre Markos University" is my real original work and all sources of materials used in this thesis have been acknowledged. It has not previously formed on the basis for the award of any Degree, Diploma of any University or other institution.

Mr. Friew Amare

Signature:-……

Date: ……

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PROTEIN SUPPLEMENTS AND THEIR EFFECT ON PHYSICAL FITNESS QUALITIES OF MEN HANDBALL TEAM PLAYERS IN THE CASE OF DEBRE MARKOS UNIVERSITY

ABSTRACT

Dietary protein supplements offer a convenient way to ensure that athletes consume quality protein in their diet and meet their protein needs. The research focus over recent years has been to determine whether different types of protein have varying effects on the physiological, hormonal and immunological responses to different type of training. Isolate of soy protein and whey protein are commonly used protein sources supplements that certainly provide adequate amounts of protein in the diet for better exercise performance. Therefore, in this study, the researcher want to investigate the effect of Whey and Soy protein supplements on men athlete's physical fitness qualities in reference to Debre Markos University Handball team. Comprehensive sampling was used to to select samples. Twenty six male subjects were randomly assigned to two experimental and one control group. Experimental groups were taken 25g isolate whey protein for EWG and 25g isolate soy protein to ESG for eight weeks after each training program. However, the control group did not taken any protein types. All data were analyzed using t-test and one-way measures analysis of variance. Twenty-six participants were completed the eight week study. Subjects in the EWG and ESG were shown significant difference between means in the strength and power than the CT group. Except EWG no significant difference between means were observed in the endurance performance in the ESG and placebo group after the experimental period.

Key words: Handball team, Whey protein supplement, Soy protein Supplement, Strength, Power, Endurance

TABLE OF CONTENTS

Contents **pages**

GRADUATE STUDIES APPROVAL PAGE ii
DECLARATION OF AUTHORSHIP iv
ACKNOWLEDGEMENTS v
ABSTRACT vi
TABLE OF CONTENTS vii
LIST OF TABLES x
LIST OF FIGURES xii
ABBREVIATIONS xiii
CHAPTER ONE 1
1. INTRODUCTION 1
1.1. Background of the Study 1
1.2. Statement of the Problem 3
1.3. Objectives of the Study 5
1.3.1. General Objective of the Study 5
1.3.2. Specific Objectives of the Study 5
1.4. Research Hypotheses 6
1.5. Delimitations of the Study 6
1.6. Limitations of the study 6
1.7. Significances of the Study 7
1.7. Operational Definition of Terms 7
CHAPTER TWO 8
2. REVIEW OF LITERATURE 8
2.1. Dietary Protein Supplementation 8
2.2. Protein Sources and Quality 9
2.2.1. Protein Sources 9
2.2.2. Quality of Whey and Soy Proteins 11
2.3. Whey Protein 12
2.3.1. Isolate Whey Protein and Health 14
2.3.2. Isolate Whey Protein and Effects on Exercise 15
2.4. Soy Protein 17

2.4.1. Isolate Soy Protein and Health 18
2.4.2. Isolate Soy Protein and Effects on Exercise 19
2.5. Physical Qualities of Handball Players 21
2.5.1. Anaerobic Power 22
2.5.2. Intermittent High Intensity (Anaerobic) Endurance 22
2.5.3. Maximal Hand Grip Muscle Strength 23
CHAPTER THREE 24
3. RESEARCH METHODS 24
3.1. Study Area 24
3.2. Research Design 24
3.3. Population, Sample and Sampling Technique 24
3.3.1. Population of the study 24
3.3.2. Sampling Techniques and Samples 25
3.4. Source of Data 25
3.5. Inclusion and Exclusion Criteria of participants 25
3.5.1. Inclusion Criteria 25
3.5.2. Exclusion Criteria 25
3.6. Supplementation Protocol 25
3.7. Data Collection Instruments 27
3.7.1. Maximal Hand Grip Muscle Strength Test 27
3.7.2 Anaerobic Power Field Based Test 28
3.7.3. Intermittent High Intensity (Anaerobic) Endurance Field Based Test 30
3.8. Data Quality Control 32
3.9. Data Analysis 32
3.10. Ethical Consideration 32
CHAPTER FOUR 33
4. RESULTS AND DISCUSSIONS 33
4.1. Results of the Study 33
4.2. Discussions 44
CHAPTER FIVE 50
5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS 50
5.1. Summary 50
5.2 Conclusion 51

5.3 Recommendations 52
REFERENCE I
APPENDIX 1: PARTICIPANTS INFORMED CONSENT FORM XIII
APPENDIX 2: ETHICAL CLEARANCE XIV
APPENDIX 3: ROW DATA II
APPENDEX 4; YO-YO IR1 TEST RECORD SHEET III
APPENDEX 5; ANOVA AND POST HOC TEST RESULT IV

LIST OF TABLES

Table number	Table titles	page
Table 1	Soy and whey protein quality adapted from Report of an FAO Expert Consultation (FAO, 2013)……...…..	11
Table 2	Yo-Yo intermittent recovery level 1 test protocol adopt from Souhail et al. (2010). ……..	31
Table 3	Demographic characteristics of subjects ……..	33
Table 4	Descriptive Statistics of physical qualities with independent variables.	34
Table 5	Independent sample t-test results of Maximal hand grip muscle strength between experimental whey protein supplement group and control group..	35
Table 6	Independent sample t-test results of Maximal hand grip muscle strength between experimental soy protein supplement group and control group.....	36
Table 7	Independent sample t-test results of peak anaerobic power between experimental whey protein supplement group and control group. ..…….....	37
Table 8	Independent sample t-test results of peak anaerobic power between experimental soy proteins supplement group and control group. …….....	37
Table 9	Independent sample t-test results of intermittent high intensity endurance between experimental whey protein supplement group and control group..	38
Table 10	Independent sample t-test results of intermittent high intensity endurance between experimental soy protein supplement group and control group….	39
Table 11	One way ANOVA results of hand grip muscular strength between experimental whey protein supplement group, soy protein supplement group and control group …….....	39
Table 12	One way ANOVA multiple comparisons table of hand grip muscular strength between experimental whey protein supplement group, soy protein supplement group and control group. ……..	40

Table 13 One way ANOVA results of peak anaerobic power output between experimental whey protein supplement group, soy protein supplements group and control group. ……..……..... 41

Table 14 One way ANOVA multiple comparisons table of peak anaerobic power output between experimental whey protein supplement group, soy protein supplement group and control group. ……...…….. 42

Table 15 One way ANOVA results of intermittent high intensity endurance between experimental whey protein supplement groups, soy protein supplement group and control group. …….. 43

Table 16 One way ANOVA multiple comparisons table of intermittent high intensity endurance between experimental whey protein supplement group, soy protein supplement group and control group…….. 44

LIST OF FIGURES

Fig. No	Fig. Title	Page
Fig. 1	Golden standard whey and soy protein supplements…...…….……25	
Fig. 2	Hand grip muscular strength test. ……...……..27	
Fig. 3	Vertec Vertical Jump test Meter. ……..28	
Fig. 4	Sayers power output estimation formula from vertical jump test …. ……..29	
Fig. 5	Yo-Yo intermittent recovery level 1 test. ……...…… ……..30	
Fig. 6	Bangsbo formula to estimate VO_2 max from YYIR1 test result. … ……..…..31	

ABBREVIATIONS

CG: Control Group

CI: Confidence Interval

DMU: Debre Markos University

ESG: Experimental Soy Protein Group

EWG: Experimental Whey Protein Group

FAO: Food and Agriculture Organization

Fig.: Figure

IHIEP: Intermittent high intensity endurance performance

ISP: isolate soy protein

IWP: isolate whey protein

KG: Kilogram

MHGMS: Maximal hand grip muscle strength

PAPw: Peak Anaerobic Power

PDCAAS: Protein Digestibility Corrected Amino Acid Score

SE: Standard Error

USA: United States America

USFDA: United States Food and Drug Administration

WHO: World Health Organization

WPC: whey protein concentrate

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Handball is a sport grouped into ball games sport, which is characterized by fast pace defensive and offensive action with the objective of scoring and preventing goals. To score goals, the offensive players attempt to establish an optimal position for the throwing player by fast movements over short distances performing powerful changes in direction, one-on-one action against defensive players and passing the ball using different offensive tactics. And also to prevent goals the defender team players obstruct an opponent team players with or without the ball (Janusz Czerwinski, 1997).

To describe this play, team handball is a complex intermittent game, which requires players to have well developed aerobic and anaerobic capacities (Heaton et al., 2017). Situations during the game always change between standing and walking, jogging and moderate running, jumping, sprinting and fast forward, sideward, and backward (Wagner H. et al., 2014) and also, a specific high level of endurance is important to keep up a high level of play during the entire game (2×30min) (Bautista et al., 2016).

In this highly complex sport, successful performance required depends on a series of basic fitness qualities such as strength, power, speed, agility, balance and endurance (Sporiš, Vuleta, & Milanović, 2010). Because of this, during periods of intensive training and competition, the recommended intake of energy and macronutrients must be needed, especially for carbohydrates (CHO) and proteins, in order to maintain appropriate body mass, optimal recovery of muscle glycogen stores, and tissue construction and regeneration (Heaton et al., 2017; Kalpakçioğlu, 2008). Due to the need such type of diet, in addition to the row foods, dietary supplements can play a meaningful role in helping athletes consume the proper amount of calories in their diet. While it is true those most dietary supplements need for athletes have little scientific data supporting their potential ability to enhance training and/or performance. It is also true that a type of nutrients

and/or dietary supplements have been shown to help improve performance and/or recovery for different type of training (Massimo Negro, 2014).

Dietary supplements are dietary substances that have extra diet by increasing the total dietary intake products that bears or contains one or more of the following dietary ingredients: vitamins, minerals, amino acids, herbs or others. They are intended to be taken by mouth, in forms such as tablet, capsule, powder, or liquid (Benjamin, `2006).

Athletes are especially at risk for muscle loss due to heavy exercise that leads to increased nitrogen loss and muscle breakdown (negative nitrogen balance), and the preventable solution is consumption of adequate amounts of protein (Omar Aljaloud, 2015). Dietary protein supplements offer a convenient way to ensure that athletes consume quality protein in their diet and meet their protein needs. The research focus over recent years has been to determine whether different types of protein have varying effects on the physiological, hormonal and immunological responses to different type of training. In addition, different researches have examined whether timing of protein intake and type of protein play a role in promoting net protein synthesis accretion and training adaptations (Massimo Negro, 2014).

As a protein source, whey, casein, soy protein isolate, egg white, meat protein has the highest attainable protein score of 1.0, as determined by the internationally accepted PDCAAS method (Protein Digestibility Corrected Amino Acid Score). PDCAAS has been adopted by the Food and Drug Administration in the USA by the recommendation of the World Health Organization (WHO) a score of 1.0 means that 100% of the essential amino acids are found that are required by human beings (Hoogenkamp, 2004). Isolate of soy protein and whey protein are commonly used protein sources supplements that certainly provide adequate amounts of protein in the diet for better muscle building (Omar Aljaloud, 2015). Because of this the researcher select the two utmost protein supplements one from animal source that is whey isolated protein supplement and the other one is soy protein that is from plant source.

Isolate whey protein is animal product protein, found in cow milk. It is extracted and isolated by removing all of the fat and carbohydrates. IWP is rapidly digested and

contains a high content of branched chain and essential amino acids. It is a popular dietary protein supplement intended to provide improved muscle strength, body composition, decrease fat mass and facilitate recovery of force production between repeated bouts of exercise in individuals due to a greater compliment of essential amino acids and branched chain amino acids as a result of greater biological value (Tang, et al, 2009). As like as IWP, isolate soy protein is a high-quality protein that provides all of the essential amino acids that needed for protein synthesis. It is extracted and isolated from soya bean, to remove all of the fat and carbohydrate contents. Some have suggested that, when used as the sole source of protein, soy protein products could be limiting in methionine. However, methionine supplementation of soy protein products in an adult diet is not usually necessary because, at levels normally produced in their body. soy protein products supply more than an adequate amount of essential amino acids for adults exclusively methionine (Endres, 2001). ISP can be used by vegetarian athletes as a good alternative to protein products from egg, meat and dairy foods to meet their protein needs (Rosenbloom, 2009).

1.2. Statement of the Problem

The nutritional requirements for different sports are determined by the game's rules, duration and frequency of competition, training phase, number of players and substitutions that are allowed during competition (Holway & Spriet, 2011). Furthermore, performance and recovery outcomes, as well as physiological adaptations, are unique to the modality of training primarily employed. Like other team sports, handball is characterized by an intermittent pattern of activity, with periods that requires aerobic and anaerobic metabolism as an energy source alternating with bursts of highly intense activity for a long period of time (Bautista et al., 2016; Póvoas et al., 2014; Holway & Spriet, 2011). Especially, most endurance trainings increased nitrogen loss and muscle breakdown (negative nitrogen balance) leads to muscle loss and the preventable solution is consumption of adequate amounts of protein (Omar Aljaloud, 2015; Burd, 2009). And also whether engaging in aerobic or anaerobic demanded sports, protein supplementation may have the potential to enhance or complement exercise-induced physiological responses (Packer et al., 2017).

Protein supplements suggest in a convenient way to ensure that athletes consume quality protein in their diet and meet their protein needs rather than using only raw protein content diets. The best of author's knowledge on searching Google scholar and other Google search engine no research indicated that what type and how much nutritional supplements used by Ethiopian athletes. Whereas, those elite athletes in the developed countries use nutrition supplements in addition to the row foods to improve their athletic performance (G. Mauro & Pasquale, 2008). On the other hand fasting season in Ethiopia especially Orthodox Church followers is the strictest of any Church, with 180 mandatory fasting days (Ethiopian Orthodox Church, 2018). Due to this and for vegetarian athletes, soy protein as a good alternative to protein products from meat and dairy foods to meet their protein needs (Rosenbloom, 2009).

ISP and IWP are commonly used protein sources that certainly provide adequate amounts of protein in the diet for better muscle building (Omar Aljaloud, 2015). Because both protein supplements are a high quality protein that provides the entire essential amino acids needed for protein synthesis (Hoffman & Falvo, 2004a).

Different studies focused over a years has been to determine whether soy or whey protein supplementation have varying effects on the physiological, hormonal and immunological responses for resistances training athletes and soccer players (Davies & Jakeman, 2018; Giezenaar et al., 2018; Mark Messina et al., 2018; Kraemer et al., 2013; Boutin et al., 2012; Mortensen et al., 2012; Lollo et al., 2011; Chad M. Kerksick et al., 2006; T.Parker & J.Goodrum, 1990). However to the best of author's knowledge by search using Google scholar, PubMed, science direct and other Google search engines, scarcity of studies were found on the effects of isolate whey and isolate soy types of protein supplementation on strength, power and intermittent high intensity endurance parameters of handball players. Furthermore, there are limited evidences on comparative effect of plant and animal sourced proteins, particularly IWP and ISP.

Due to this, the researcher needs to investigate the issue (testing the effect of soy protein and whey isolate protein supplements on selected physical qualities of Handball players).

1.3. Objectives of the Study

1.3.1. General Objective of the Study

The general objective of this study is to investigate the effect of protein supplements on men athlete's physical fitness qualities in reference to Debre Markos University Handball team.

1.3.2. Specific Objectives of the Study

- To find out the effect of isolate whey protein supplement on power, endurance and muscular strength performance of Debre Markos University Handball team players.
- To evaluate the effect of isolate soy protein supplement on power, endurance and muscular strength performance of Debre Markos University Handball team players.
- To identify whey or soy protein supplement has more effect on power, endurance and muscular strength performance of Debre Markos University Handball team players.

1.4. Research Hypotheses

The following are null hypothesis of the research

H_O. Whey protein supplement has not produce significant improvement on power, endurance and muscular strength performance of Debre Markos University Handball team players.

H_O. Soy protein supplement has not a significant effect for the improvement on power, endurance and muscular strength performance of Debre Markos University Handball team players.

H_O. There is no significant difference between whey protein and soy protein supplements to produce improvement on power, endurance and muscular strength performance of Debre Markos University Handball team players.

1.5. Delimitations of the Study

This study was conducted on handball players of Debre Markos University which is one of second generation university in Ethiopia. The research investigate by intervene isolate soy protein and isolate whey protein supplements to test effects on different three variables (anaerobic power, intermittent high intensity endurance and maximal hand grip muscle strength performance) on male athletes. The intervention time was been from December 01/2019 up to January 30/2020 G.C which was the preparation period of a team.

1.6. Limitations of the study

Food habits and the way of life style, which could influence on the results, could not be controlled by the researcher. However the researcher and a coach were provide orientation about these aspects to the subjects. Since the subjects and test items were not able to take all the measurements alone. Therefore the researcher took help from some assistants, the researcher considered those things as a limitation of this study.

1.7. Significances of the Study

The results of this study will be helpful in giving information and highlights about the effect of protein supplements especially isolate soy and isolate whey protein supplements for handball coaches, trainee and for sport nutrition professionals who serves in handball teams. Besides the finding of this study, it would help for the researchers to be aware and knowledgeable of the processes involve in the all-round research. It would help as a future reference for more studies.

1.7. Operational Definition of Terms

- Whey protein (WP): is refers to a dietary supplement and food ingredient created by separating components of milk by removing of all fat and carbohydrate to get higher percentage of pure protein (Mingruo Guo, 2019).
- Soy protein (SP): refers to the most highly refined soy bean products that are prepared from removing most of the non-protein components (Endres, 2001).
- Anaerobic power performance (APP): is refers to a capacity to release the maximum force in shortest time: fewer than 10 seconds. (Bhadu & Singh, 2016)
- Intermittent high intensity (anaerobic) endurance performance (IHIEP): is refers to the body's potential for long-term performance at high intensity, is well recognized in elite handball and other ball game sport (Iaia & Bangsbo, 2010).
- Maximal hand grip muscle strength (MHGMS): is refers to the maximum isometric strength of the hand and forearm muscles (I. Zapartidis et al., 2009).

CHAPTER TWO

2. REVIEW OF LITERATURE

2.1. Dietary Protein Supplementation

Dietary supplements are substances that might use to add nutrients to the diet and it comes in the form of pills, capsules, powders, gel tabs, extracts, or liquids. They might contain vitamins, minerals, fiber, amino acids, herbs or other plants, or enzymes (Omar Aljaloud, 2015).

Athletes may have elevated physiological protein requirements, to maintain adequate protein synthesis and energy production, as well as sufficient immune function and good gut integrity in the multi-stress conditions of goal-directed, frequent, intensive and/or prolonged exercise routines. To meet these specific nutritional requirements, athletes use several types of protein supplements in addition to their row food (Benjamin Caballero , MD, 2006).

Protein is needed to support the repair of damaged body tissues and the building of new proteins in response to the training stimulus (Westerterp-Plantenga, Nieuwenhuizen, Tomé, Soenen, & Westerterp, 2009). Research has indicated that people undergoing intense training may need additional protein in their diet to meet protein needs. People who do not ingest enough protein in their diet may exhibit slower recovery and training adaptations. Protein supplements offer a suitable way to ensure that athletes consume quality protein in the diet and meet their protein needs (M. Negro, Rucci, Buonocore, Focarelli, & Marzatico, 2013).

According to the current literature we know that the addition of protein (about 20 -30 g) before and/or after resistance training can increase protein synthesis and gains in lean mass beyond normal adaptation (Kraemer et al., 2013; Hoffman et al., 2007; Chad M. Kerksick et al., 2006; Brown et al., 2004).

In case of athlete’s nutritional requirement, it appears that protein supplementation by increasing daily protein intake to a level higher than 12-15% of sedentary people diet. It

will be too high for most athletes (Goyena, 2019). Protein intake for any endurance athlete will thus be sufficient as long as the diet is well composed and contains a variety of protein sources such as lean meat, fish, dairy products, eggs and vegetable protein. Supplementation may be warranted for athletes who compete in weight classes, intensive training and weight reduction programmers (Hayley Daries, 2012). Also vegetarian athletes, who consume low energetic and low protein diets (Nieman, 2008). Or athletes, who for any reason are unable to ingest sufficient protein, may benefit from some protein supplementation with the goal to achieve their nutritional protein need per day. Ingesting a moderate amount (20-30 g) of protein powder can do this (Goyena, 2019). Consequently, nitrogen consumed in excess of that which is immediately required to support the 'optimal' rates of amino acid utilizing functions outlined above will ultimately result in urea production, and oxidation of the resulting carbon skeletons.(Phillips, 2012)

2.2. Protein Sources and Quality

2.2.1. Protein Sources

Protein is available in a variety of dietary sources. These include foods of animal and plant origins as well as the highly marketed sport supplement industry. Proteins from both vegetable and animal sources, including milk, egg and soy are the main types of protein supplements. In the following section proteins from both vegetable and animal sources, including whey, egg, soy and others will be explored.

2.2.1.1. Milk proteins

Milk proteins have undergone extensive research related to their potential roles in augmenting adaptations from exercise training(Hartman et al., 2007). For example, consuming milk following exercise has been demonstrated to accelerate recovery from muscle damaging exercise(Cockburn et. al., 2010), increase glycogen replenishment (Wojcik et al., 2001), improve hydration status (Cockburn et al., 2010; Watson P, et.al, 2008), and improve protein balance to favor synthesis, ultimately resulting in increased gains in both neuromuscular strength and skeletal muscle hypertrophy (Hartman et.al., 2007). Moreover, milk protein contains the highest score on the PDCAAS rating system,

and in general contains the greatest density of leucine. Milk can be fractionated into two protein classes, casein and whey(Jäger et al., 2017). Contains both ‘‘fast’’ and ‘‘slow’’ proteins with 80% casein and 20% whey protein. Whey protein is water soluble, mixes easily, and is rapidly digested. In contrast, casein is water insoluble, coagulates in the gut and is digested more slowly than whey protein. Casein also has intrinsic properties such as opioid peptides, which effectively slow gastric motility (Wilson J, 2006).

2.2.1.2. Egg proteins

Egg protein is often thought of as an ideal protein because its amino acid profile has been used as the standard for comparing other dietary proteins (Wilson J, 2006). Due to their excellent digestibility and amino acid content, eggs are an excellent source of protein for athletes. While the consumption of eggs has been criticized due to their cholesterol content, a growing body of evidence demonstrates the lack of a relationship between egg consumption and coronary heart disease, making egg-based products more appealing (Stampfer et al, 1999). One large egg has 75 kcal and 6 g of protein, but only 1.5 g of saturated fat while one large egg white has 16 kcal with 3.5 g of protein and is fat-free. Research using eggs as the protein source for athletic performance and body composition is lacking, perhaps due to less funding opportunities relative to funding for dairy. Egg protein may be particularly important for athletes, as this protein source has been demonstrated to significantly increase protein synthesis of both skeletal muscle and plasma proteins after resistance exercise at both 20 and 40 g doses. Leucine oxidation rates were found to increase following the 40 g dose, suggesting that this amount exceeds an optimal dose (Hida et al., 2012).

2.2.1.3. Soy protein

Soy is the most widely used vegetable protein source. However, when the more accurate PDCAAS scale is used, soy protein was reported to be equivalent to animal protein with a score of 1.0, the highest possible rating (Hasler, 2002). Soy’s quality makes it a very attractive alternative for those seeking non-animal sources of protein in their diet and those who are lactose intolerant. Soy is a complete protein with a high concentration of BCAA’s (Hoffman & Falvo, 2004a).

2.2.1.4. Whey Protein

Whey protein is one of the most popular (and most advertised) protein supplements to athletes. Researchers believe that the key could be the amino acid leucine, one of the BCAAs. Whey protein has the highest amount of BCAAs of any protein. After exercise, leucine stimulates signaling pathways to stimulate muscle protein synthesis (Rosenbloom, 2009).

2.2.2. Quality of Whey and Soy Proteins

Determining the effectiveness of a protein is accomplished by determining its quality and digestibility. Quality refers to the availability of amino acids that it supplies, and digestibility considers how the protein is best utilized (Hoffman & Falvo, 2004a).Amino acids ingested by humans exist in a combined form as dietary proteins from both animal and vegetable sources. Not all proteins in the diet have the same nutritional value, since they contain different proportions of essential amino acids (Lanham & Collins, 2011). A variety of whole protein foods (milk, meat, fish, egg, and vegetables) exist, and a number of component protein powders (eg, casein, whey, soy) are readily available (Lemon, Berardi, & Noreen, 2002). It does appear that protein from animal sources is an important source of protein for athletes. With a proper combination vegetable proteins may provide similar benefits as protein from animal sources. Maintenance of lean body mass though may become a concern (Lemon et al., 2002).

Characteristically, all dietary animal protein sources are considered to be complete proteins. That means a protein that contains all of the essential amino acids. Proteins from vegetable sources are incomplete in that they are generally lacking one or two essential amino acids except soy protein (FAO, 2013). Digestibility characteristics of protein source, whey, casein, soy protein isolate, egg white, meat protein has the highest attainable protein score of 1.0, as determined by the internationally accepted PDCAAS method (Protein Digestibility Corrected Amino Acid Score). PDCAAS has been adopted by the Food and Drug Administration in the USA by the recommendation of the World Health Organizations (FAO/WHO). A score of 1.0 means that 100% of the essential amino acids are found that are required by human beings (Hoogenkamp, 2004).

Net protein utilization measures protein quality by calculating the nitrogen used for tissue formation divided by the nitrogen absorbed from food. This product is multiplied by 100 and expressed as a percentage of nitrogen utilized. The biological value provides a measurement of how efficient the body utilizes protein consumed in the diet (Hoffman & Falvo, 2004a). The protein efficiency ratio (PER) determines the effectiveness of a protein through the measurement of animal growth. This technique requires feeding rats a test protein and then measuring the weight gain in grams per gram of protein consumed (Kalpakçioğlu, 2008).

Table 1. *Soy and whey protein quality adapted from Report of an FAO Expert Consultation* (FAO, 2013)

Protein type	Protein efficiency	Net protein utilization	Protein digestibility corrected Amino Acid Score
Whey protein	3.2	92	1.00
Soy protein	2.2	74	1.00

This data is displayed before the foods make an additional filtering process removes more fat and carbs, further concentrating the protein. Protein isolate powders contain ≥90% protein both isolate soy and whey proteins (Endres, 2001; Parker, 2004).

2.3. Whey Protein

Milk gets follow the process (coagulation and curd removal) of cheese manufacturing. From this fluid, whey proteins are separated and refined using different techniques yielding different concentrations of whey proteins. All of the ingredients of whey protein provide high levels of the essential and branched chain amino acids. The bioactivities of these proteins possess many beneficial properties as well (Phillips, 2012). Whey protein is most recognized for its applicability in sports nutrition. And also, it is also riches in vitamins and minerals (Jäger et al., 2017).

Whey protein also contains a high concentration of branched chain amino acids (BCAA) that are important for their role in the maintenance of tissue and prevention of catabolic actions during exercise (Williams, 2005).

The majority of whey is processed into whey powder. Whey derivates are used as ingredients in a wide range of products, with the lowest valuable ones used predominately by the feed industry. Derivate with higher value, such as whey protein concentrate (WPC) and whey protein isolates (WPI) are produced for the food, cosmetic and pharmaceutical sectors. Upon concentration or isolation, water, lactose, fat and ash or dust are removed from the total solids of liquid whey in varying amounts. This yields a wide variety of whey protein products, including WPC, which contains 25-80% protein, and WPI containing ≥90% protein (Massimo, 2014).

There are three different forms of whey protein that result from various processing techniques used to separate whey protein. They are whey powder, whey concentrate, and whey isolate (Massimo, 2014). Whey protein powder has many applications throughout the food industry. As an additive it is seen in food products for dairy, bakery, confectionery, and snack products. Whey powder itself has several different varieties including sweet whey, acid whey (seen in salad dressings), demineralized (seen primarily as a food additive including infant formulas) (Mingruo Guo, 2019).

Whey protein concentrate removes the water, lactose, ash, and some minerals. In addition, compared to whey isolates whey concentrate typically contains more biologically active components and proteins that make them a very attractive supplement for the athlete (Mingruo, 2019).

Isolate whey protein contains >90% protein, 4–6% water and the remaining 4–6% of the ingredient is a combination of fat, lactose, and ash. Because of their high protein purity, IWP is used extensively in nutritional supplements, sports and health drinks, and protein-fortified beverages (Parker, 2004). Ion exchange chromatography is one of the methods used in the manufacture of IWPs. It provides an additional level of selectivity above membrane processing, because factors other than molecular size determine protein

absorption. Because of the high protein content, IWP functions as a water-binding, gelling, emulsifying and foaming agent (Garba & Kaur, 2014).

2.3.1. Isolate Whey Protein and Health

Diets absolutely high in protein may promote renal damage via excretion of nitrogenous waste products generated from protein metabolism, thereby increasing glomerular pressure and hyper filtration(Westerterp et al., 2009) .

Whey protein is among the best studied supplements in the world, and for good reason. It has a very high nutritional value, and scientific studies have revealed numerous health benefits (Arnarson, 2017).

The research done by 158 people, those who were given whey lost significantly more body fat and showed a greater preservation of lean muscle compared to subjects consuming the control beverage (Frestedt et al., 2008). Another research results were indicated that the use of whey protein concentrate in cancer treatment (Bounous G., 2000). A study, published in The British Journal of Nutrition gave whey supplements to 70 overweight men and women for 12 weeks and measured a number of parameters, such as lipid and insulin levels. They found that there was a significant decrease in total cholesterol and LDL cholesterol at week 12 in the whey group compared with the casein group (Pal & Dhaliwal, 2010). And a research published in the International Dairy Journal found that beverages that were supplemented with whey protein significantly reduced blood pressure in patients with hypertension; their risk of developing heart disease or stroke was also lower (Fluegel et al., 2010).

But in contrast some people who are allergic to milk may be specifically allergic to whey. In moderate doses, whey protein does not typically cause any adverse events. However, consuming very high doses can cause: stomach pains, cramps, reduced appetite, nausea, headache and fatigue (Nordqvist, 2017). There are different whey isolate protein manufacturers in the world. The present study indicated that the WP-USA supplements showed better nutritional quality, as evaluated through contents of TP, α-LA, β-LG, and free BCAA. This data emphasize the necessity to improvement the oversight with respect to the quality of WP supplements sold on the market, regardless of the origin of the

manufacture. Therefore, periodic evaluation of products and verification of labeling are advisable in order to guarantee product quality for consumers (Almeida & Conte-Junior, 2016).

2.3.2. Isolate Whey Protein and Effects on Exercise

Whey protein (WP) supplementation has recently gained popularity amongst athletes as it is reported to improve athletic performance. Whey protein is a popular dietary protein supplement intended to provide improved muscle strength and body composition due to a greater compliment of essential amino acids and branched chain amino acids and to result in greater biological value (Burke et al., 2001). Whey protein supplement is a cornerstone to enhance athletic performance and optimize training adaptations including the consumption of a balanced, nutrient and energy dense diet. whey protein supplementation has shown to reduce oxidative stress through increasing endogenous glutathione production and improve compromised gut health associated with intense exercise (Kerksick et al., 2018).

While the majority of the literature regarding the effects of protein intake on performance has focused on anaerobic activities, more recent work has examined its role on endurance activities, but this has mostly been absent from the most recent reviews. But the research done by whey protein by soccer player was concluded that supplementation immediately after training sessions during the competitive period is beneficial and safe, as well as capable of sustaining or even increasing muscle mass and endurance by testing yo-yo and 3000m intermittent endurance test (Lollo et al., 2011). A meta-analysis on efficacy and safety of whey protein supplements on vital sign and physical performance among athletes indicates that IWS had better efficacy among all the supplements (Lam et al., 2019). Some studies have demonstrated that endurance exercise results in increased leucine oxidation with acute endurance exercise, there is an increase in the proportion of carbohydrate oxidation and leucine oxidized which is found in whey protein (Bowtell et al., 1998).

A research done by basketball players in order to observe the effects of whey protein powder on hematological indexes of players majoring in physical education in the

basketball training, the authors divided the players randomly into a control group and a nutrition group. Athletes complete the 30 minutes quantitative exercise using cycle ergometer respectively before the trial and after one month trial. The results showed that after taking whey protein powder, the hemoglobin, read blood cell, hematocrit of nutrition group was significantly higher that the control group. This suggests that in high-intensity training, taking whey protein powder can cause changes of HB, RBC and HCT in human body. So whey protein powder can improve exercise capacity, and has anti-fatigue effect (Taylor & Dugan, 2015).

In the other hand many researches amplify effects of whey protein supplement on anaerobic athletic performance like power, speed and others. the presence of protein in rehydration beverages can enhance intestinal fluid uptake, aiding in rehydration (JB, 1998) and that BCAA consumption during endurance exercise may improve time trial performance and peak power output while improving markers of immune health and attenuate serotonin levels, subsequently resulting in a delay of central fatigue (Wesley et al., 2016). The findings revealed that Meta-analysis done by a total of 333,257 research articles has shown that WPS has significantly overall increased in the anaerobic performance of athletes compared to the control group (without WPS) (Lam et al., 2019).

The effect of protein supplementation on athletic performance and hormonal changes was examined for 12 week in 21 experienced college football players were randomly assigned to either a protein supplement (PR; n = 11) or a placebo (PL; n = 10) group. whey protein supplementation appeared to significantly augment lower body strength development, similar upper body strength, anaerobic power and lean tissue changes (Chad M. Kerksick et al., 2006). Another research examined if 8 weeks of whey protein (WP) supplementation improved body composition and performance measures in NCAA Division III female basketball players. Subjects were assigned to consume 24 g WP (n = 8; age, 20 ± 2 years; height, 170±6cm; weight, 66.0 ± 3.1 kg) or 24 g of malt dextrin (MD) (n = 6; age, 21 ± 3 years; height, 169 ± 6 cm; weight, 68.2 ± 7.6 kg), the WP group presented greater gains in 1-repetition maximum (1RM) bench press (+4.9 kg) compared with the MD group (+2.3 kg) ($p < 0.05$) (Taylor et al., 2015).

Intact protein supplements, all essential amino acids have been shown to be beneficial for the exercising individual by increasing the rates of muscle protein synthesis, decreasing muscle protein degradation, and possibly aiding in recovery from exercise. In summary, increasing protein intake using whole foods as well as high-quality supplemental protein sources can improve the adaptive response to training (Jäger et al., 2017).

2.4. Soy Protein

Soy is the most widely used vegetable protein source. The soybean, from the legume family, was first chronicled in China in the year 2838 B.C. and was considered to be as valuable as wheat, barley, and rice as a nutritional staple. Soy's popularity covered several other countries, but did not gain notoriety for its nutritional value in The United States until the 1920s. The American population consumes a relatively low intake of soy protein (5g/ day) compared to Asian countries (Hoffman & Falvo, 2004b). Although cultural differences may be partly responsible, the low protein quality rating from the PER scale may also have influenced protein consumption tendencies. However, when the more accurate PDCAAS scale is used, soy protein was reported to be equivalent to animal protein with a score of 1.0, the highest possible rating. Soy's quality makes it a very attractive alternative for those seeking non-animal sources of protein in their diet and those who are lactose intolerant. Soy is a complete protein with a high concentration of BCAA's (Wilcker & Waggle, 1978).

The soybean can be separated into three distinct categories; flour, concentrates, and isolates. Soy flour can be further divided into natural or full-fat (contains natural oils), defatted (oils removed), and lecithinated (lecithin added) forms (Endres, 2001). Of the three different categories of soy protein products, soy flour is the least refined form. It is commonly found in baked goods. Another product of soy flour is called textured soy flour. This is primarily used for processing as a meat extender (Hettiarachchy et al.,1998).

Soy protein products categorize into three major groups. These groups are based on protein content, and range from 40% to over 90%. All three basic soy protein product groups (except full-fat and partially defatted extruded-expelled flours) are derived from

defatted flakes. They are: soy flours and grits, soy protein concentrates, and soy protein isolates (Endres, 2001). Soy flours and grits are prepared by crushing and screening soybean flakes either before or after removal of the oil. Their protein content is in the range of 40 to 54% (Endres, 2001).

Soy protein concentrates are prepared from dehulled and defatted soybeans by removing most of the water-soluble, non-protein constituents. They contain at least 65% protein on a moisture-free basis. Isolates soy protein are the most highly refined soy protein products commercially available. They represent the major protein fraction of the soybean. Soy isolates are prepared from dehulled and defatted soybeans by removing most of the non-protein components. They contain > 90% protein on a moisture-free basis (Endres, 2001; Hoogenkamp, 2004).

2.4.1. Isolate Soy Protein and Health

Some research suggests that adding soy protein to your diet may provide a variety of health benefits. For example, soy foods appear to have positive effects on heart health. In a review of 35 studies, soy consumption lowered "bad" LDL cholesterol and raised "good" HDL cholesterol (Tokede et al., 2015). Some observational and case-controlled studies link soy intake to a reduced risk of breast cancer (Yamamoto et al., 2003).

When discussing soy's role in men's health, some observational studies indicate that consuming soy foods may reduce the risk of prostate cancer in older men (Yamamoto et al., 2003).

Studies indicate that high-protein diets can result in weight loss, even without limiting calories or nutrients. However, the evidence is mixed regarding the relationship between soy protein and weight loss. Some studies show that soy protein can boost weight loss as effectively as animal-based proteins (Due, 2004). A 12-week weight loss study found similar results with soy protein powder. Participants received soy-based or non-soy-based meal replacements. Both resulted in an average weight loss of 17.2 pounds (7.8 kg) by the end of the study (Beavers et al., 2016).

But in the drawback of soy protein, soy protein contains phytates, also known as anti-nutrients. These reduce the availability of iron and zinc in soy protein (Al-Wahsh et al., 2005; Hurrell et al., 1992). Studies on the impact of soy on thyroid function in humans suggest it may not have a significant impact. In a review of 14 studies, soy had little to no effect on thyroid function, and the authors concluded that people with hypothyroid disease do not need to avoid soy as long as their iodine intake is adequate (Messina & Redmond, 2006).

Nonetheless, soy protein can serve as a good source of plant-based protein for people who don't consume animal proteins, including vegetarians and vegans, allowing them to secure the significant benefits of this nutrient (Montgomery, 2003).

2.4.2. Isolate Soy Protein and Effects on Exercise

Isolate soy protein as like as other dietary protein has an important regulator of protein metabolism in skeletal muscle and maintaining of lean body mass. The research done by 40 male athletes, participants performed a cycling test on an electrically braked cycle ergometer. Findings indicate that no change in total body mass, resistance training led to significant improvements in fat free mass (pre- vs. post- training: 21.9+5.1 vs. 20.7+4.5 kg; p50.01) ac companied by significant reductions in waist circumference (97.9+7.9 vs. 96.7+6.3 cm; p50.05) and fat mass (21.9+5.1 vs. 20.7+4.5 cm; p50.01); the training induced changes in body composition were more pronounced in the soy supplement group (Deibert et al., 2011).

The study was done to evaluate the possible valuable effects of soy protein supplementation and exercise training on mice. The mice received a soy protein (3.8 g/kg BW) by oral for four weeks, and the exercise performance (forelimb grip strength and exhaustive swimming time) was evaluated. The study demonstrated that a combination of ISP supplementation and exercise reduced fatigue and improved bone function in mice (Lin & Huang, 2018).

Hoffman et. al (2007) reported that soy protein group had significantly greater improvement in 1-RM squat strength compared to placebo, but no difference in the magnitude of improvement was seen between the groups for the 1-RM bench press and

Wingate anaerobic power test was no significant PRE to POST changes in peak power, mean power, fatigue rates or total work occurred in either group (Hoffman et al., 2007).

A meta-analysis was conducted to compare the effect of supplementation with soy protein to animal protein supplementation on strength and lean body mass in response to resistance training. The result showed that, there was no significant effect of whey or soy alone (n = 5) on lean body mass change and no differences between groups ($\chi 2 = 0.00$, $p = .96$). Strength and LBM both increased significantly in the "other protein" and the soy groups (n = 9), but there were no between-group differences (bench: $\chi 2 = 0.02$, $p = .88$; squat: $\chi 2 = 0.78$, $p = .38$; and lean body mass: $\chi 2 = 0.06$, $p = .80$). The results of this meta-analysis indicate that soy protein supplementation produces similar gains in strength and LBM in response to RET as whey protein (Mark Messina et al., 2018). In contrast a meta-analysis done by untrained individuals, consuming supplemental protein likely has no impact on lean mass and muscle strength during the initial weeks of resistance training (Pasiakos et al., 2014). However, as the duration, frequency, and volume of resistance training increase, soy protein supplementation may promote muscle hypertrophy and enhance gains in muscle strength in both untrained and trained individuals. Tracy G. Anthony et. al. investigated the research to compare the early response of skeletal muscle protein synthesis and translation initiation following the ingestion of different protein One hour after endurance exercise, serum insulin concentrations in experimental carbohydrate group, experimental soy protein, and experimental whey protein supplement group were greater than in control group (P, 0.05); the concentration in experimental whey was greater than in experimental carbohydrate, with that in experimental soy intermediate. Serum concentrations of branched-chain amino acids in experimental soy and experimental whey group were higher than in experimental carbohydrate group, but serum leucine and isoleucine in experimental whey were higher than in experimental soy group (P, 0.05). Nevertheless, both experimental soy and experimental whey protein group promoted the fractional rate of skeletal muscle protein synthesis significantly more than in experimental carbohydrate group. (Anthony et al., 2007)

Evidence also suggests that protein supplementation may accelerate gains in both aerobic and anaerobic power (Pasiakos et al., 2014). In addition of anaerobic performance soy protein supplement improves athlete's endurance capacity. For instance the study was compared soy protein to whey protein in terms of the short-term recovery of muscle protein synthesis and translation initiation in skeletal muscle following endurance exercise. The result of the study indicated that both soy protein and whey protein promoted general muscle protein synthesis and formation after treadmill exercise (Anthony et al., 2007).

2.5. Physical Qualities of Handball Players

Handball is very complex sport where successful performance depends on a number of basic abilities in particular agility, strength, power, speed and intermittent endurance. Creativity in combination with speed and strength as well as coordination makes this sport very attractive but tough to play (Granados, Izquierdo, & Iba, 2007). The manifestation of these characteristics and their mutual interactions interest a large number of scientists around the world and force them to research this field (Srhoj & Rogulj, 2002).

Some studies have revealed that handball is a very complex sports activity where successful performance depends on a number of basic motor abilities, mostly on the ability of movement, explosive strength (power), basic strength of the trunk, and psychomotor speed (Katic & Srhoj, 2008; Srhoj et al., 2002). Situation performance is predominantly determined by explosive strength because the conditions of handball impose the need of maximal jumping, throwing or sprint performance (Katic et al., 2008).

Gorostiaga and his teammates investigate the relationships between the physical conditioning markers monitored during the course of a season and the quantitative assessments of training and competition in male handball players. The findings show that linear relationships between periods devoted to strength or high-intensity endurance training and changes in endurance running, throwing velocity, and muscle power output of the lower extremities (Gorostiaga & Izquierdo, 2006).

2.5.1. Anaerobic Power

It is a capacity to release the maximum force in shortest time quickly within 10 seconds (Bhadu & Singh, 2016). A power player is not only strong but generates the force quickly. Handball players make a powerful shooting or power of legs when jumping to words for an attack. The power is also known as strength ability of the player is during the use of power in shooting, throwing & passing. The players are used to have develop strength their body muscles of both lower and upper extremities. For example: weight lifting, sprinting, shooting and jumping (Pennington, 2014). Lower extremity of handball player anaerobic power performance can be measured by vertical jump (VJ), horizontal jump (HJ), counter movement jump and others (Karadenizli, 2016).

2.5.2. Intermittent High Intensity (Anaerobic) Endurance

Anaerobic endurance is the ability of performing organs and systems, in particular the central nervous system, to function of intermittent (high intensity exercise with stop and start at intervals) activities for a long period of time. Sports such as soccer require performance of anaerobic activities like sprinting and acceleration This principle also applies to handball and basketball that have similar but not exactly the same movement requirements predominately due to the respective size of playing areas (Kemi et al., 2003).

Activities in team sports such as soccer, handball and basketball are comprised of varying explosive movement patterns (like forward, side-to-side and backward), runs at different intensities (e.g., from jogs to sprints), kicks, tackles, turns, jumps, and sustained forceful muscle contractions to control the ball against defensive pressure (Bangsbo, Iaia, & Krustrup, 2008a). For this reason importance of anaerobic endurance, i.e. the body's potential for long-term performance at high intensity, is well recognized in team sports (Kemi et al., 2003). A research showed that prolonged motor performance at relatively high speeds leads to fatigue, a consequential decrease in technique and an unsatisfactory final outcome. For this reason anaerobic endurance development, which helps to delay the onset of fatigue as well as reduces the fatigue effect, can be beneficial (Bangsbo et al., 2008a; Kemi et al., 2003).

The anaerobic endurance capacities of handball players are measured through Yo-Yo intermittent recovery test level I. The players were administered with ten minutes of warming up. Then players were asked to line up in front of twenty meter marked area with cones. The tester instructs the subjects to run half way and return to the starting point when the sound signal produced from music player. The tester keeps recording the distance covered by the players (Wood R, 2018).

2.5.3. Maximal Hand Grip Muscle Strength

Even if handgrip is an vital measurement of general health and is used as an predictor of the well function as it is referred to as one of the best accurate clinical methods of estimating children's strength (Groslambert, Nachon, & Rouillon, 2002). Furthermore, the handgrip of experienced arm in handball is important for the holding and throw of the ball. On the other hand, the ball's velocity in handball is a major racing skill and looks to be a distinction between the most and the less successful athletes, especially during developing ages (Zapartidis et al., 2009). The maximal handgrip strength of the dominant hand will measure using a hand dynamometer (Tsakalou & Zapartidis, 2015; Zapartidis et al., 2009).

CHAPTER THREE

3. RESEARCH METHODS

3.1. Study Area

The study was conducted at Debre Markos University Handball team. Debre Markos University is a public research university found in the city of Debre Markos, Ethiopia. It was established in 2005 and has been growing fast. Debre Markos is found in the North-west of the capital city of Ethiopia, Addis Ababa, at a distance of 300 kms.

3.2. Research Design

This thesis was followed quantitative researches approach because it involves the generation of data in quantitative form which can be subjected to rigorous quantitative analysis.

And also it was used true experimental design with control group and two experimental groups which were taken whey or soy protein with barley juice in blind manner. But all three groups participate equally in preparation period of handball training.

3.3. Population, Sample and Sampling Technique

3.3.1. Population of the study

Male beginner Handball team players who train in Debre Markos University were the source population for this study. Twenty eight athletes were taken as populations who were participated in Debre Markos University Handball team.

3.3.2. Sampling Techniques and Samples

Comprehensive sampling was used based up on the predetermined parameters from beginner athletes of Debre Markos University Handball team, who were train in 2019 / 2020 training year.

3.4. Source of Data

Primary sources of data were collected through the administration of experimental process by recording post test result of an aerobic power, high intensity intermittent endurance and hand grip strength from the samples were taken from athletes who are a member of Debre Markos University Handball team.

3.5. Inclusion and Exclusion Criteria of participants

3.5.1. Inclusion Criteria

- Having no major identified health problems (cardiovascular disease, kidney etc.).
- Subjects must be institutional meal service users
- Not being in injured status
- Who sign his informed consent

3.5.2. Exclusion Criteria

Out of the eligible subjects, those who could not come due to long rest days, being injured or sickness were being excluded from the study.

3.6. Supplementation Protocol

After selecting with the above criteria**,** all groups were engage in preparatory phase of handball training. In this period athletes were concentrating on general conditioning and sport specific exercises. This includes exercise to develop aerobic and anaerobic capacity of an athlete (Bompa & Haff, 2009; Paul Gamble, 2006). Before start taking supplement all athletes were tested their physical qualities (anaerobic power, handgrip strength and anaerobic endurance) based on test protocol. Then, soy protein supplement group (ESG)

and whey protein supplement group (EWG) were taken protein supplement after each training session for eight weeks (3days/week) by professional sport nutritionist. The other group (CG) was taken a mix of 250 ml of water with 10 g sugar and 30 g barley powder and regular handball training only.

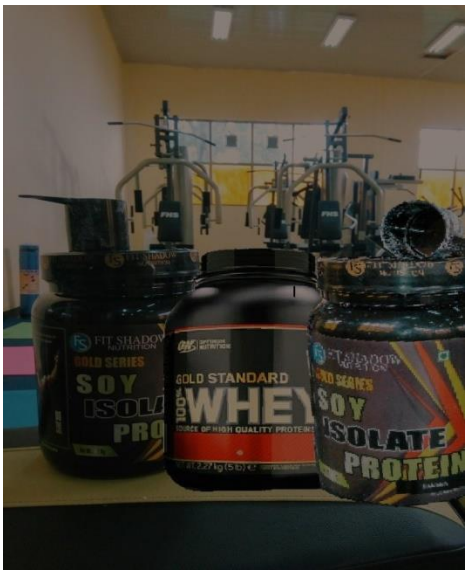

Fig 1: Golden standard whey and soy protein supplements

In the most recent reviews of protein 'requirements' for strength and endurance training athletes it was estimated, based on a meta- analytic regression, that a daily intake of ,1·3 g protein/ kg/d is required for the athlete to remain in nitrogen balance (Phillips, 2012). This bought of protein is not gain only from supplements; it includes recommended daily allowance of protein from raw diet (0.8 g protein/ kg/d). The rest of protein was gained from high-quality protein per kg of body weight, or an absolute dose of 20–30 g (Goyena, 2019; Smith et al., 2017). The level of protein supplementation is decided upon an average recommendation for high performance team sport athletes (25g) (Jäger, Kerksick et al., 2017; Rodriguez & Langley, 2009; Chad M. Kerksick et al., 2006; Brown et al., 2004) of US product and approved by USFDA (FDA, 1999). Supplements mix by 250 ml of water with 10g sugar and 30 g barley powder to ensure that experimental groups and placebo were similar in energy content, taste, texture, and appearance, during the treatment period. Supplements was taken within 30 minute (Jäger, Kerksick, et al., 2017)

after the exercise to get a maximum function of proteins in a single-blind manner during the experimental period (Lemon et al., 2002).

3.7. Data Collection Instruments

Subjects were familiarizing with the test procedures prior to the initial assessment to reduce learning effects. Measurements were undertaken before and after the intervention. To reduce the potential effect of cumulative fatigue on dependent variables outcomes, athletes had taken two day of rest between the last training session and first measurement session. Tests were complete in a single day by the same investigators and assistances, after one rest from physical activity. Warming up was an obligatory before starting a test.

3.7.1. Maximal Hand Grip Muscle Strength Test

The objective of this test is to measure the maximum isometric strength of the hand and forearm muscles. Handgrip strength is important for any sport in which the hands are used for catching, throwing or lifting(Chittibabu, 2014). Maximal isometric hand grip force was record using a handheld hand grip dynamometer (Lafayette Instrument, Lafayette IN, USA) and it is a valid instrument to measure handball players hand gripping strength (Zapartidis et al., 2016 Palamas et al., 2015; Visnapuu & T.Jürimäe, 2007). Provides gripping power up to 113kgs, Division: 100 gs, Unit: kg. The subject holds the dynamometer by dominant hand to be tested; they did it at a standing position with the dynamometer set parallel to the body. In this position, the player was asked to exert maximal grip force without arm or wrist movement. The position of the hand remained constant in a downward direction and the palm did not flex at the wrist joint.

When ready the subject squeezes the dynamometer with maximum isometric effort, which was maintained for about 5 seconds. No other body movement is allowed. The subject should be strongly encouraged to give a maximum effort. The best result from three trials for dominant hand was recorded, with at least 30 seconds recovery between each effort. Record was on the hand grip dynamometer in terms of kilogram (kg).

Fig. 2: Hand grip muscular strength test

3.7.2 Anaerobic Power Field Based Test

The most common field test which is the vertical jump test is used to evaluate anaerobic fitness improvements. It has been stated the vertical jump test is a more true power test used to measure both vertical jumping distance and power output (Stauffer, 2001). The height that is achieved on the vertical jump has a direct correlation with the amount of force that is produced by muscle fibers(Stauffer, 2001). In the vertical jump protocol a subject assumes a bent knee preparatory position, pauses and then jump vertically as high as possible.

Fig. 3: *Vertec Vertical Jump test Meter*

The Vertec Vertical Jump Meter was used as a valid vertical jump test (Buckthorpe, & Folland, 2012). It comprises plastic swivel vanes arranged in half-inch increments attached to a wired metal pole that was adjusted for each participant's reach height. The test requires participants to use their dominant hand to displace the highest possible plastic blade with an overhead arm swinging motion at the apex of their jump. Jump height was determined as the number of blades displaced above the metal pole and converted from inches to centimeters. All jumps were performed by facing the vanes at a distance of 10 cm from the post, with their dominant shoulder aligned with the end of the blades. The test material was measured the nearest to half inch.

It is useful to convert the score to units of power or work because a heavier person jumping the same height as a lighter one has to do much more work to move a larger mass. The Sayers Equation (Sayers et al., 1999) also estimates peak power output.

PAPw = (60.7 x jump height (cm)) + (45.3 x body mass (kg)) - 2055

Fig. 4: *Sayers power output estimation formula from vertical jump test*

Anaerobic Power output from the vertical jump defined as its SI unit that is in watt (Sayers et al., 1999).

3.7.3. Intermittent High Intensity (Anaerobic) Endurance Field Based Test

The assessment of the ability to perform high- intensity exercise for prolonged periods may be considered of importance in team handball for fitness assessment and talent selection (Souhail et al., 2010; Bangsbo et al., 2008a).

Hermassi souhail was done direct validity of Yo-Yo IR1 test. It may be considered as a team handball test relevant for the assessment of intermittent high-intensity endurance in young male team handball players(Souhail et al., 2010). Krustrup et al. proposed an intermittent high-intensity test, the Yo-Yo intermittent recovery test (Yo-Yo IR1), that consisted of performing 2 x 20 m shuttle running bouts (40 m) interspersed with 10m recovery until exhaustion at progressive speeds (Table 2) dictated by prerecorded audio cues.

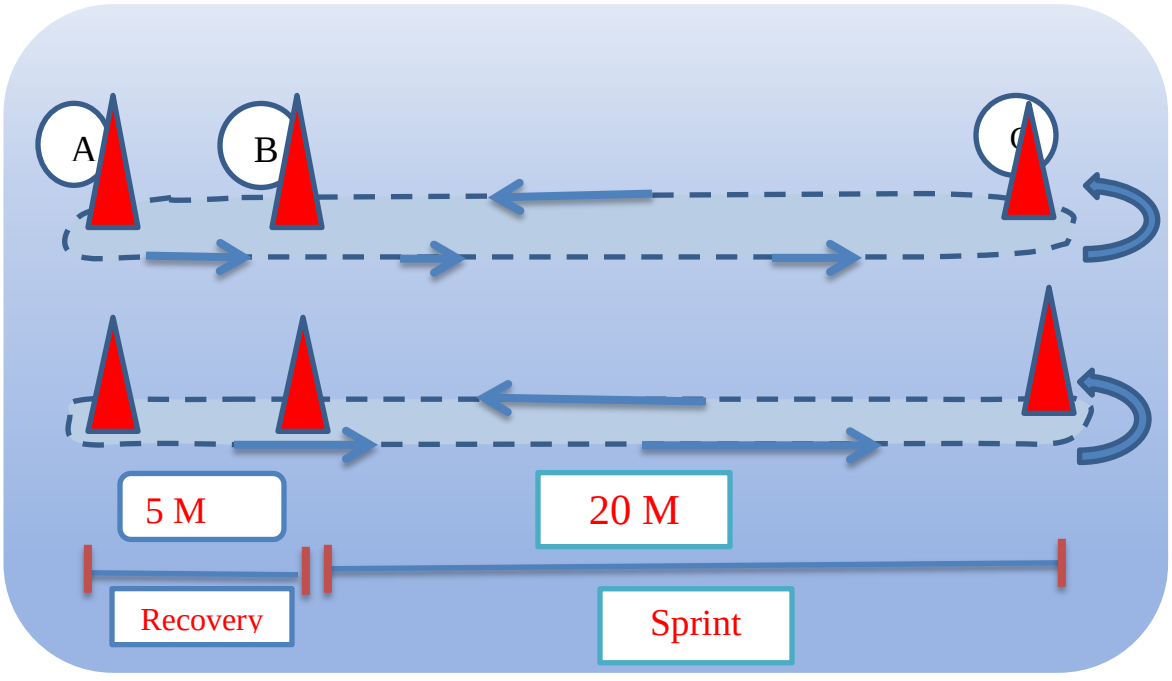

Fig. 5: *Yo-Yo intermittent recovery level 1 test*

The Yo-Yo intermittent recovery test consists of repeated 2 X 20 meter runs back and forth between the starting, turning, and finishing line at a progressively increased speed

controlled by audio bleeps from a tape recorder. Audio cues of the Yo-Yo IR1 test were recorded on a CD from https://www.theyoyotest.com and broadcasted using Sony ZS-RS60BT MP3, CD, and Radio Player. Between each running bout, the subjects have a 10 second active rest period, consisting of 2 X 5 m of jogging. When the subjects twice have failed to reach the finishing line in time, the distance covered is recorded and represents the test result. The total distance covered during the Yo-Yo IR1 (including the last incomplete shuttle) was considered as testing score. In the present study, we were used the Yo-Yo intermittent recovery test, level 1, which consists of different running bouts at different speed.

Table 2. *Yo-Yo intermittent recovery level 1 test protocol adopt from Souhail et al.(2010)*

Stage	Test Results (Speed Level: Shuttles)	total distance (m)	VO_{2max} (ml/kg/min)
1	**5:1**	**40**	**36.7**
2	**9:1**	**80**	**37.1**
3	**11:1**	**120**	**37.4**
4	**11:2**	**160**	**37.7**
5	**12:1**	**200**	**38.1**
6	**12:2**	**240**	**38.4**
7	**12:3**	**280**	**38.8**
8	**13:1**	**320**	**39.1**
9	**13:2**	**360**	**39.4**
10	**13:3**	**400**	**39.8**
11	**13:4**	**440**	**40.1**
12	**14:1**	**480**	**40.4**

The test was performed on handball court, marked by cones, having a width of 2 m and a length of 20 m. Another cone placed 5 m behind the finishing line marked the running distance during the active recovery period. All subjects were familiarizing to the test by giving pretest training about the test producers.

Results on this test correlate with aerobic fitness scores, and converted to a VO_2 max score by using the following formula (Bangsbo, Iaia, & Krustrup, 2008b), which is:

$$VO_{2max}\ (ml/kg/min) = distance\ run\ (m) \times 0.0084 + 36.4$$

Fig. 6: *Bangsbo (2008) formula to estimate VO_2 max from YYIR1 test result*

3.8. Data Quality Control

To maintain the quality of the data, one days training was given to one nutritionist, coaches and trainees about the objective of the study, supplement procedures and ethical issues prior to the pre-test. In addition, daily checkup and follow up was done by the researcher. When at the time of weight measurement, the scales were regularly checked and adjust to zero after each measurement in addition to this, after five participants measure the measuring equipment was calibrated by known weight, 2KG waited iron. Supplements and other substance which were given to the players were measured by a cup unit which are calibrating by gram. The collected data was checked for completeness and consistencies by investigator. The data was interred to epi data version 3.1 and finally export to SPSS to make a better analysis.

3.9. Data Analysis

Descriptive statistics was calculating the mean and standard deviations, in which the data was analyzed by SPSS statistical software package. After collecting data on those parameters as muscular strength, intermittent high intensity endurance and anaerobic power from the experimental and controlled group; it was analyzed through ANOVA. The rest of other objectives were analyzed by independent t-test. Statistical significance was considered as p-value $\leq$ 0.05. Relate the pretest scores for the control and experimental groups to see the groups which have homogeneity characteristics of participants and then compute the post test scores for the objectives of the research.

3.10. Ethical Consideration

An ethical clearance and official letter was obtained from ethical clearance committee of Debre Markos University Health Science College. Subjects were fully informed about the study in their mother language with including sign consent form. Prior to the study temporal identification numbers were allocate to each participant rather than writing their name in order to ensure confidentiality.

CHAPTER FOUR

4. RESULTS AND DISCUSSIONS

This chapter deals with the analysis of posttest data collected from two experimental and one control groups under the study. The purpose of this study was to investigate the effect of protein supplements on selected physical fitness qualities among Debre Markos University male handball players. The physical fitness qualities selected for this study were power, endurance and strength. Post-tests were taken from all experimental and control groups after 8 weeks of protein supplement intervention, and the scores were recorded. The collected data were analyzed using ANOVA and independent t-test to analyze post-test results of experimental and control groups.

4.1. Results of the Study

From 28 subjects who were initially enrolled 26 participants were completed the study. Two subjects were eliminated from this study due to being injured at the posttest period. Pretest data of each variable were tested by ANOVA and the researcher were affirmed that there was no significance difference between each group by age, height weight and BMI.

Table 3. *Demographic characteristics of subjects*

Group..	**N**	**AGE**	**Height**	**Weight**	**BMI**
		Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
EWG	8	20.88 ± 1.95	1.64 ± 0.06	55.17 ± 2.90	20.37 ± 1.49
ESG	9	22.11 ± 2.20	1.66 ± 0.05	56.40 ± 3.32	20.38 ± 0.61
CG	9	20.11± 1.36	1.70 ± 0.10	57.63 ± 3.58	19.92 ± 1.00

Key: - EWG=Experimental whey protein group, ESG=Experimental soy protein group, CG= Control group, BMI= body mass index.

As shown from the above table, descriptive characteristics of 26 study subjects from Debre Markos University handball team of age (EWG=20.80 ± 1.75, ESG=21.81 ± 2.30, CG= 20.60 ± 2.01), height (EWG=1.64 ± 0.06, ESG= 1.66 ± 0.05, CG= 1.70 ± 0.10), body weight (EWG= 55.17 ± 2.90, ESG= 56.40 ± 3.32, CG= 57.63 ± 3.58) and body mass index (EWG=20.42 ± 0.88, ESG =20.36 ± 0.58, CG= 20.28 ± 0.64). The participants were homogeneous in terms of their age and anthropometric characteristics such as body height, weight and body mass index (BMI).

Table 4. *Descriptive Statistics of physical qualities with independent variables*

Physical qualities	Group		
	EWG	ESG	CG
	Mean± Std.	Mean± Std.	Mean± Std.
MHGMS	56.98 ± 1.80	56.26 ± 3.56	51.99 ± 4.75
PAPw	4405.10 ± 317.70	4545.17 ± 235.46	3752.90 ± 428.79
IHIEP	50.48 ± 0.46	48.01 ± 1.16	47.76 ± 0.49

Key: EWG=Experimental whey protein group, ESG=Experimental soy protein group, CG= Control group, MHGMS= Maximal hand grip muscle strength, PAPw= Peak Anaerobic Power, IHIEP: Intermittent high intensity endurance performance.

The above table shows the post test results of Maximal hand grip muscle strength, peak Anaerobic Power and intermittent high intensity endurance performance score for all experimental and control groups. As shown in the table 4 the post test score of maximal hand grip muscle strength of EWG, ESG and CG were found to be 56.98 ± 1.80 kg, 56.26 ± 3.56 kg and 51.99 ± 4.75 kg respectively. In case of Peak Anaerobic Power of EWG, ESG and CG mean score found to be 4405.10 ± 317.70watt, 4545.17 ± 235.46watt and 3752.90 ± 428.79watt respectively. The post test score of intermittent high intensity endurance performance EWG and ESG found to be 50.48 ± 0.46 ml/kg/min and 47.99 ± 1.10 ml/kg/min respectively. Whereas, CG mean value was 47.76 ± 0.49 ml/kg/min. This implies that, there was mean difference between the groups, yet it is impossible to tell here if the differences are statistically significant. Hence ANOVA and independent sample t-test used as comparing the post test scores between groups based on dependent

variables and which was computed to examine whether this number show statistical difference between group mean scores. The independent sample t-test and ANOVA results were presented in the table as follows.

Table 5: *Independent sample t-test results of Maximal hand grip muscle strength between experimental whey protein supplement group and control group.*

	Levene's Test		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	SE	95% CI Lower	Upper
MHGMS	4.723	.056	2.604	15	.020	4.659	1.79	.85	8.47

Key: df= degree of freedom, SE= standard error deference, CI= confidence interval deference

If there was difference in maximum hand grip muscular strength between experimental whey protein group and control group, Independent sample t-test was run to determine. Inspection of Q-Q plots revealed that maximum hand grip muscular strength was normal distributed for both groups and that there was homogeneity of variance as assessed by levene's Test for Equality of variances. Therefore an independent t-test was run on the with a 95% confidence interval (CI) for the mean difference. It was found that after eight week intervention, maximum hand grip muscular strength in the whey protein supplement group (56.98 ± 1.80 kg) were significantly higher than the control group (51.99 ± 4.75 kg) ($t(15) = 2.604$, $p = 0.02$, 95% CI [.85, 8.47].

Table 6: *Independent sample t-test results of Maximal hand grip muscle strength between experimental soy protein supplement group and control group.*

	Levene's Test		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed	Mean Diff.	SE	95% CI Lower	Upper
MHGMS	.629	.439	2.01	16	.041	3.292	1.953	-.84934	7.4337

Key: SE= Std. Error Difference, df= degree of freedom, t= computed test statstics, sig.(2-taild)= p-value,

Independent sample t-test was done to determine If there was difference in maximum hand grip muscular strength between experimental soy protein supplement group and control group,. Inspection of Q-Q plots revealed that maximum hand grip muscular strength was normal distributed for both groups and that there was homogeneity of variance as assessed by levene's Test for Equality of variances. Therefore an independent t-test was run on the with a 95% confidence interval (CI) for the mean difference. It was found that after eight week intervention, maximum hand grip muscular strength in the soy protein supplement group (56.26 ± 3.56 kg) were significantly higher than the control group (51.99 ± 4.75 kg) ($t(16) = 2.01$, $p = 0.04$, 95% CI [-0.84, 7.43].

Table 7: *Independent sample t-test results of peak anaerobic power between experimental whey proteins supplement group and control group.*

	Levene's Test		**t-test for Equality of Means**						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	SE	95% CI	
								Lower	Upper
PAPw	.224	.643	3.439	15	.004	647.73	188.36	246.23	1049.238

Key: SE= Std. Error Difference, df= degree of freedom, t= computed test statistics, sig. (2-taild) = p-value, CI= Confidence Interval of the Difference

Independent sample t-test was done to determine if there was difference in peak anaerobic power output between experimental whey protein supplement group and control group. All assumptions of independent t-test were tested and passed them. Therefore an independent t-test was run on the with a 95% confidence interval (CI) for the mean difference. Hence, ($P < 0.05$) after eight week intervention, peak anaerobic power in the whey protein supplement group (4405.10 ± 317.70 watts) were significantly higher than the control group (3752.90 ± 428.79watts) ($t(15) = 3.439$, $p = 0.04$, 95% CI [246.23, 1049.2].

Table 8: *Independent sample t-test results of peak anaerobic power between experimental soy proteins supplement group and control group.*

	Levene's Test		**t-**test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	SE	95% CI	
								Lower	Upper
PAPw	1.67	.214	4.45	16	.001	703.23	157.74	368.82	1037.64

Key: SE= Std. Error Difference, df= degree of freedom, t= computed test statistics, sig. (2-taild) = p-value, CI= Confidence Interval of the Difference

If there was difference in peak anaerobic power output between experimental soy protein supplement group and control group, Independent sample t-test was done to determine. All assumptions of independent t-test were tested and passed them. Therefore an independent t-test was run with a 95% confidence interval (CI) for the mean difference. Hence, (P <0.05) It was found that after eight week intervention, peak anaerobic power in the soy protein supplement group (4545.17 ± 235.46 watts) were significantly higher than the control group (3752.90 ± 428.79watts) (t(16) = 4.45, p = 0.00, 95% CI [368.82, 1037.64].

Table 9: *Independent sample t-test results of intermittent high intensity endurance between experimental whey protein supplement group and control group.*

	Levene's Test		t-test for Equality of Means						
	F	Sig.	t	Df	Sig. (2-tailed)	Mean Diff.	SE	95% CI Lower	95% CI Upper
IHIEP	.985	.337	11.322	15	.001	2.5946	.2291	2.11	3.08

Key: SE= Std. Error Difference, df= degree of freedom, t= computed test statistics, sig. (2-taild) = p-value, CI= Confidence Interval of the Difference

An independent sample t-test was conducted to compare intermittent high intensity endurance between experimental whey protein supplement group and control group. All assumptions of independent t-test were tested and passed them. Therefore an independent t-test was run on the with a 95% confidence interval (CI) for the mean difference. It was found that after eight week intervention, intermittent high intensity endurance in the whey protein supplement group (50.48 ± 0.46 ml/kg/min) were significantly higher than the control group (47.76 ± 0.49 ml/kg/min) (t(15) = 11.322, p = 0.01, 95% CI [2.11, 3.08]. Since p= 0.00 is less than our chosen significance level $\alpha < 0.05$, we can reject the null hypothesis, and conclude that the mean VO_2 max for whey protein supplement group and control group is significantly difference.

Table 10: *Independent sample t-test results of intermittent high intensity endurance between experimental soy protein supplement group and control group.*

	Levene's Test		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Diff.	SE	95% CI Lower	Upper
IHIEP	3.185	.093	.613	16	.058	.261	.426	-.642	1.164

Key: IHIEP= intermittent high intensity endurance performance, SE= Std. Error Difference, df= degree of freedom, t= computed test statistics, sig. (2-taild) = p-value, CI= Confidence Interval of the Difference.

If there was difference in mean of intermittent high intensity endurance between experimental soy protein supplement group and control group, Independent sample t-test was done to determine. All assumptions of independent t-test were tested and passed them. Therefore an independent t-test was run with a 95% confidence interval (CI) for the mean difference. Hence, ($P < 0.05$) there was not a significant difference after eight week intervention, mean of intermittent high intensity endurance in the soy protein supplement group (48.01 ± 1.16 ml/min/kg) with mean of control group (47.76 ± 0.49 ml/kg/min) ($t(16) = 0.613$, $p = 0.058$, 95% CI [-0.642, 1.164].

Table 11: *One way ANOVA results of maximum hand grip muscular strength between experimental whey protein supplement group, soy protein supplement group and control group*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	99.064	2	49.532	3.908	.035
Within Groups	291.517	23	12.675		
Total	390.582	25			

Key: df = degree of freedom, F= variance statistics, sig = significance level

The output of the ANOVA table shows that the significance value was 0.035 (p = 0.035), which is below 0.05. Therefore there is a statistically significant difference in the maximum hand grip strength between two different protein supplement and control group.

Table 12: *One way ANOVA multiple comparisons table of hand grip muscular strength between experimental whey protein supplement group, soy protein supplement group and control group*

(I) group category	(J) group category	Mean Difference (I-J)	SE	Sig.	95% CI	
					Lower Bound	Upper Bound
EWG	ESG	1.36722	1.72992	.713	-2.9651	5.6995
	CG	4.65944*	1.72992	.033	.3271	8.9918
ESG	EWG	-1.36722	1.72992	.713	-5.6995	2.9651
	CG	3.29222*	1.67827	.045	-.9107	-2.4952
CG	EWG	-4.65944*	1.72992	.033	-8.9918	-.3271
	ESG	-3.29222	1.67827	.045	-7.4952	.9107

Key: * = The mean difference is significant at the 0.05 level, SE = standard error, CI = confidence interval, Sig. = significance level

The Tukey post hoc test was used for conducting post hoc test on a one –way ANOVA. Based on the result of multiple comparison table, there is a statistically difference in maximum hand grip strength between the groups that took EWG and CG (P = 0.33) as well as between ESG and CG (P = 0.045). However, there were no differences between the groups that took ESG and EWG (P= 0.713).

There was a statistically difference between groups as determined by one- way ANOVA (F(2,23) =3.908, P = 0.035. A Tukey post hoc test revealed that the maximum hand grip strength was whey protein supplement group (56.98 ± 1.80 kg, p = 0.033) and soy protein supplement group (56.26 ± 3.56 kg, p= 0.045) respectively statistically significantly higher compared to the control group (51.99 ± 4.75 kg). There was no statistically significant difference between the whey protein supplement group and soy protein supplement group (p = 0.713).

Table 13: *One way ANOVA results of peak anaerobic power output between experimental whey protein supplement group, soy protein supplement group and control group*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2711055.527	2	1355527.763	11.918	.001
Within Groups	2616013.389	23	113739.713		
Total	5327068.915	25			

Key: df = degree of freedom, F= variance statistics, sig = significance level

The output of the ANOVA table shows that the significance value was 0.01 (p = 0.00), which is below 0.05. Therefore there is a statistically significant difference in the peak anaerobic power output between whey protein supplement group, soy protein supplement and control group.

Table 14: *One way ANOVA multiple comparisons table of peak anaerobic power output between experimental whey protein supplement group, soy protein supplement group and control group*

(I) group category	(J) group category	Mean Difference (I-J)	SE	Sig.	95% CI	
					Lower Bound	Upper Bound
EWG	ESG	-55.49250	163.875	.939	-465.891	354.90
	CG	647.73750*	163.875	.002	237.33	1058.13
ESG	EWG	55.49250	163.875	.939	-354.90	465.89
	CG	703.23000*	158.982	.001	305.08	1101.37
CG	EWG	-647.73750*	163.875	.002	-1058.13	-237.33
	ESG	-703.23000*	158.982	.001	-1101.37	-305.08

Key: * = The mean difference is significant at the 0.05 level, SE = standard error, CI = confidence interval, Sig. = significance level

Multiple comparison tables display the Tukey post hoc test on a one –way ANOVA for conducting post hoc test. Based on the result of multiple comparison table, there is a statistically difference in peak anaerobic power output between the groups that took EWG and CG (P = 0.02) as well as between ESG and CG (P = 0.001). However, there were no differences between the groups that took SEG and WEG (P= 0.939).

There was a statistically difference between groups as determined by one- way ANOVA ($F(2,23) = 11.918$, $P = 0.00$. A Tukey post hoc test revealed that the peak anaerobic power output was whey protein supplement group (4405.10 ± 317.70 watt, p = 0.002) and soy protein supplement group (4545.17 ± 235.46 watt, p= 0.001) respectively statistically significantly higher compared to the control group (3752.90 ± 428.79 watt). There was no statistically significant difference between the whey protein supplement group and soy protein supplement group (p = 0.939).

Table 15: *One way ANOVA results of intermittent high intensity endurance between experimental whey protein supplement groups, soy protein supplement group and control group*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.933	2	16.966	27.481	.001
Within Groups	14.200	23	.617		
Total	48.133	25			

Key: df = degree of freedom, F= variance statistics, sig = significance level

The output of the ANOVA table shows that the significance value was 0.01 (p = 0.01), since, p < 0.05 is significance. Therefore there is a statistically significant difference in the peak anaerobic power output between whey protein supplement group, soy protein supplement and control group.

Table 16: *One way ANOVA multiple comparisons table of intermittent high intensity endurance between experimental whey protein supplement group, soy protein supplement group and control group*

(I) group category	(J) group category	Mean Difference (I-J)	SE	Sig.	95% CI Lower Bound	Upper Bound
EWG	ESG	2.333*	.38180	.001	1.3772	3.2895
	CG	2.594*	.38180	.001	1.6385	3.5508
ESG	EWG	-2.333*	.38180	.001	-3.2895	-1.3772
	CG	.2613	.37040	.763	-.6663	1.1889
CG	EWG	-2.594*	.38180	.001	-3.5508	-1.6385
	ESG	-.261	.37040	.763	-1.1889	.6663

Key: * = The mean difference is significant at the 0.05 level, SE = standard error, CI = confidence interval, Sig. = significance level

Multiple comparison tables display the Tukey post hoc test on a one –way ANOVA for conducting post hoc test. Based on the result of multiple comparison table, there is a statistically difference in intermittent high intensity endurance between the groups that took EWG and ESG (P = 0.01) as well as between EWG and CG (P = 0.01). However, there were no differences between the groups that took ESG and CG (P= 0.763).

There was a statistically difference between groups as determined by one- way ANOVA (F(2,23) =27.481, P = 0.00. A Tukey post hoc test showed that the intermittent high intensity endurance was whey protein supplement group (50.48 ± 0.46 ml/kg/min, p = 0.00) statistically significantly higher compared to ESG (48.01 ± 1.16 ml/kg/min) control group (47.76 ± 0.49 ml/kg/min). There was no statistically significant difference between the soy protein supplement group and control group (p = 0.763).

4.2. Discussions

The purpose of this study is to investigate the effect of protein supplements on men athlete's physical fitness qualities in reference to Debre Markos University Handball team. Before start taking supplement all athletes were tested their physical qualities (anaerobic power, handgrip strength and anaerobic endurance) based on test protocol. The pretest was analyzed by ANOVA to see whether the groups mean have significant deference or not before intervention. Therefore the researcher assures that there were no significances different between groups mean in terms of three independent variables before intervention. Then, soy protein supplement group (ESG) and whey protein supplement group (EWG) were taken protein supplement after each training session for eight weeks by professional sport nutritionist. No side effects were observed due to the intervention with the protein supplementation in any of the groups in spite of the fact that the total protein intake (70 g) exceeded recommendations for endurance athletes (Rosenbloom, 2009).

After completion of intervention athletes were tested their physical qualities (anaerobic power, handgrip strength and endurance) based on preplanned test protocol. The data was calculated the mean and standard deviations, independent t-test and ANOVA. In which the data was analyzed by SPSS statistical software package. After collecting data on

those parameters; muscular strength, intermittent high intensity endurance and anaerobic power output from the experimental and controlled group; it was analyzed through ANOVA.

Based on the result of analysis the following hypothesis was rejected:

- Whey protein supplement has not produce significant improvement on power, endurance and muscular strength performance of Debre Markos University Handball team players.
- Soy protein supplement has not a significant effect for the improvement on power and muscular strength performance of Debre Markos University Handball team players.
- There is no significant difference between whey protein and soy protein supplements to produce improvement on power, and muscular strength performance of Debre Markos University Handball team players.

On the other hand the following hypothesis was accepted based on the output result of SPSS

- Soy protein supplement has not a significant effect for the improvement on endurance performance of Debre Markos University Handball team players.
- There is no significant difference between whey protein and soy protein supplements to produce improvement endurance performance of Debre Markos University Handball team players.

The finding of this study based on the result of SPSS output, each variable are discussed as follows.

The findings of the study (table-5) revealed that there were significance differences between experimental whey protein group and control group maximum hand grip muscular strength. Whey protein supplement group (56.98 ± 1.80 kg) was show significant improvement than the control group (51.99 ± 4.75 kg). This result was in line with the findings from previous meta-analyses and supports the positive effect of high

quality protein supplementation, including whey; maximize upper body muscle strength when combined with strength training intervention (Cermak et.al 2012). In contrast the research had done on effects of protein supplementation on muscular performance and resting hormonal changes in college football players result shows that no other significant strength or power differences were seen between whey protein group and placebo groups(Hoffman et al., 2007).

The result of ANOVA revealed that whey protein and soy protein supplements have significant effect on strength and power output of athletes. Results of the current meta-analysis do support such speculation as strength and lean tissue accretion in response to resistance training were similar between whey and soy protein supplementation(Brown, Disilvestro, Babaknia, A., & Devor, 2004; Candow, Burke, N.C., Smith-Palmer, T., & Burke, 2006; Denysschen, Burton, H.W., Horvath, Leddy, J.J., & Browne, 2009; Maltais, Ladouceur, J.P., & Dionne, 2016; Morton et al., 2017). In contrast the meta-regression also conducted by these Morton, including 15 studies, which investigated the influence of protein source (soy vs. whey) on change in strength, concluded it is potentially a very minor determinant(Morton et al., 2017).

In addition, eight week whey protein supplement were improve peak anaerobic power output in contrast to control group. This result was in agreement with the findings of Jay R. Hoffman et.al on ten week of whey protein supplement produce improvement on strength, power and body composition. A significant main effect on whey protein supplement groups in strength improvement was seen in 1RM bench press 125.4 ± 16.7 at Week 10 testing,) and 1RM squat 169.0 ± 25.5 Week 10 testing,(Hoffman et al., 2009). Other researcher Lemuel W. Taylor(2015) reviled that Whey protein supplementation following exercise in female collegiate basketball players improves vertical jump height and reduces fat mass compared with a placebo supplement(Taylor et al., 2015). Also some investigators have shown augmented on whey protein supplementation has been shown to significantly enhance power performance(Andersen et al., 2005), others have shown no significant differences between subjects consuming a protein supplement compared to placebo on power development of athletes (Chromiak et al., 2004; Hoffman et al., 2007)

In addition to the known role of exercise and protein supplementation on the possible gain of skeletal muscle mass(Blake BR, et.al., 2000; Roth E, 2008) our data suggest that supplementing immediately after training with whey proteins can provide significant benefit for maintaining, or even gaining muscle mass in young elite athletes undergoing intense and prolonged physical exercise. This result was in agreement with the findings of S. Ronghi (2016). The result of Ronghui showed that after taking whey protein powder, the hemoglobin and red blood cell of nutrition group was significantly higher than control group. This research suggests that in high-intensity training, taking whey protein powder can cause changes of HB, RBC and hematocrit in human body. So whey protein powder can improve exercise capacity, and has anti-fatigue effect (Ronghui, 2016). In addition Huang et. al.(2017) done a research on 12 elite male track runners showed that; the endurance performance in twelve minute run was significantly elevated possibly due to an increase in the muscle mass and amelioration of exercise(Huang, Chang, et.al., 2017). In the current study, we demonstrated that whey protein can also used for aerobic exercise for better physiological adaptation, in addition to anaerobic capacity development. However, to our knowledge there are only limited studies on the effect of whey protein supplement in “non body building” athletes such as basketball, football, handball players who engage in complex training schedules.

Our data did show an improvement in handgrip strength and vertical jump (anaerobic power) of the athletes that consumed the soy protein supplement after sport training; this did not show result in an improvement in the yoyo tests. The finding of the current research revealed that soy protein supplement group has a significant effect on hand grip muscular strength than control group.

This result was in agreement with the findings of Mark Messina and his teammates (2018) meta-analysis is consistent with the totality of evidence regarding protein supplementation and exercise. This meta-analysis identified that soy protein supplements can be viewed as sources of protein suitable for building strength and increasing lean tissue in response to sport training (Mark Messina et al., 2018). Candow and colleagues(Candow DG, Burke NC, Smith-Palmer T, 2066) studied soy protein (25-30 g) on untrained young adults and compared it with whey protein (25-30 g) and found that

both soy and whey protein increased strength and power output compared with placebo. Other researchers(Phillips SM, Harman JW, 2005) have shown that whey protein is superior to soy protein for muscle anabolism, but the results should not be interpreted that soy protein has no effect on muscle strength. Candow and colleagues (Candow DG, Burke NC, Smith-Palmer T, 2066) studied soy protein (1.2 g/kg of body weight) on untrained young adults and compared it with whey protein (also at 1.2 g/kg) and found that both soy and whey protein increased lean mass compared with placebo. Other researchers (Kaldirimci, et. al, 2015) have shown that whey protein is superior to soy protein for muscle anabolism, but the results should not be interpreted that soy protein has no effect on muscle growth. However, to our knowledge there are only limited studies on the effect of soy protein supplement in "non bodybuilding" athletes such as basketball, football, handball players who engage in complex training schedules.

Generally Researchers believe that the key could be the amino acid leucine, one of the BCAAs(Chromiak et al., 2004; G. & Jackson, 2001; G. Mauro & Pasquale, 2008; M. Negro et al., 2013; Rosenbloom, 2009; Wolinsky & Driskell, 2000). Whey and soy protein has the highest amount of BCAAs of any protein. After exercise, leucine stimulates signaling pathways to stimulate muscle protein synthesis(Gibala MJ, 2007). Norton and Layman reported that leucine serves as a crucial regulator of protein synthesis and is donor of nitrogen to alanine and glutamine, important amino acids in muscle protein synthesis(Norton LE, 2006). Researchers are excited about this ''leucine trigger,'' as it may have important implications for muscle growth for healthy athletes.

Supplements of 25g consumed immediately after the training sessions by young male athletes appeared to be a safe strategy to follow during handball training of several weeks preparation period aiming at developing their physical qualities. In view of these results and supporting literature, coaches could consider protein supplementation, particularly with the whey and soy protein hydrolyzed as a potentially beneficial nutrients for the young athlete facing intense and prolonged physical training in order to maintain strength, power and endurance all throughout. In this type of sport, training is not aimed at muscle mass building beyond an ideal point, which should be attained prior to the competitive period. According to our results 25g of protein supplement is better after

intensive preparation period training to develop athlete's strength and power. However, whey protein supplement in addition could be better than the soy proteins hydrolyzed in terms of improving high intensity endurance capacity of young handball trainees.

CHAPTER FIVE

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

This study was tried to investigate the effect of protein supplements on selected physical fitness qualities among Debre Markos University male handball players. To achieve this purpose, the researcher reviewed the available literatures in order to decide the focus of the study and methodologies. In order to attain the general objective of the study, the following specific research objectives were formulated and well achieved.

- To find out the effect of isolate whey protein supplement on muscular strength, power and endurance performance of Debre Markos University Handball team players.
- To evaluate the effect of isolate soy protein supplement on muscular strength, power and endurance performance of Debre Markos University Handball team players.
- To identify whey or soy protein supplement has more effect on muscular strength, power and endurance performance of Debre Markos University Handball team players.

Based on the above specific objectives, the hypotheses were formulated. Subjects for the study were 26 males who were participating in Debre Markos University Comprehensive sampling was used to select subjects up on the predetermined parameters. Subjects were randomly assign to two experimental and one control group. Experimental groups were taken (25g) isolate whey protein for EWG and (25g)isolate soy protein to ESG for eight week after each training program. However, the control group did not taken any protein types. Nevertheless, they did perform as equal as normal training activities as experimental group members on the regular handball training program.

All participants partook in all three physical qualities tests: hand grip muscular strength, vertical jump for anaerobic power output and yo-yo test for high intensity endurance capacity. The data was gathered from the experimental and control groups results had been organized using appropriate and relevant statistical method of analysis (Independent t-test and ANOVA).which assists to come up with findings. The collected a data was used to analyze not only different mean scores between experimental and control group but also between three groups mean values.

Generally 8 week whey protein and soy protein supplements in general preparation period for handball training had a positive effect on hand grip muscular strength and vertical jump anaerobic power output and also whey protein supplement had a tendency to improve endurance fitness performance of handball trainees not only than control group but also soy protein supplement group.

5.2 Conclusion

Based on the major finding of this study, the following points were stated as a conclusion.

- Eight weeks whey protein supplements had a positive effect on development of hand grip muscular strength physical quality of handball players.
- Eight weeks soy protein supplements had a positive effect on development of hand grip muscular strength physical quality of handball players.
- Regular eight week whey protein supplementation has greater increases in the peak anaerobic power output physical quality of handball players.
- Eight weeks soy protein supplements had a positive effect on development of power output physical quality of handball players.
- Implementing eight weeks whey protein supplements had a positive effect on development of intermittent high intensity endurance physical quality of handball players.

5.3 Recommendations

Considering the major finding and conclusion of the study it is important to state the following recommendation:

- Based on the result the researcher recommended that coaches, nutritionists and sport professionals apply whey protein and soy protein supplements at the preparation period of athletes after each training session to develop muscular strength and power output performance.
- Especially coaches, nutritionists and sport professionals need to apply whey protein supplements not only for the development of strength and power but also for the development of high intensity endurance performance of handball players.
- The coaches, sport nutritionists and sport professionals are better to apply soy protein supplements to empower muscular strength and power in fasting season. Because soy protein can used as a good alternative to protein products from meat and dairy foods to meet their protein needs for handball trainees.
- To provide a compelling argument as to the reason behind of the result, this study recommends for further research in the same area on proteins in terms of amino acids with different sex, timing of supplement, amount of supplements and some more functional variables like skills and physical qualities of handball trainees.

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APPENDIX 1: PARTICIPANTS INFORMED CONSENT FORM

Title: **P**rotein supplements and their effects on selected physical fitness qualities of men athletes: in the case of Debre Markos University Handball team

The purpose of this study will be to examine the effects of isolate soy and isolate whey protein supplements on selected physical fitness qualities of men athletes in reference to Debre Markos University Handball team. You are being asked to voluntarily participate in this research because you are a part of Debre Markos University Handball team member.

This study is going to involve the supplementation of isolate soy and isolate whey protein post to a bout of exercise, to see if it has any effect on the amount of anaerobic power, muscular strength and intermittent endurance. You will be assigned to a particular group, and this will determine if you will be given isolate soy protein group, placebo or isolate whey protein supplement group. Each supplement group of players will take 25g of protein powder mix with 30 gram of barley powder diluted in 250ml of water. Before and after start supplement each players test their initial performance based on test protocol. You will be asked to wear appropriate clothing to ensure optimal performance. You will also be asked to refrain from any additional supplementation during the time of the study. All data and producers from this study will be collected and handled in confidentially way.

Risk: Some people who are allergic to milk may be specifically allergic to whey. In moderate doses, whey and soy protein does not typically cause any adverse events. However, consuming very high doses can cause: stomach pains, cramps, reduced appetite, nausea, headache, fatigue. Consistent high doses of whey protein may also cause acne.

Benefits: In participating in this experiment, you may gain some understanding of the effect of protein supplement and its benefits it has on physical quality. If successful, you may also benefit by receiving effective exercise supplement that you can then implement in your daily exercise routine.

Confidentiality: All information concerning you will be kept private. If any information about you is published, it will be written in a way that you will not be identifiable. All

data associated with your participation in this study will be kept confidential except where noted, and as required by law. You will not be identified by your name at all.

In the event that injury occurs as a result of this research, medical treatment will be available. For more information concerning the research and research related risks or injuries, you may contact the investigator:

Name: Friew Amare

Phone no: 0947590535

Email: firew143@gmail.com

Voluntary Participation /Withdraw: Participation in this research study is entirely voluntary. You are free to decline or end participation at any time throughout this investigation. Your decision to participate or not will have no effect on your current or future interaction with the investigator.

Contact Persons: To make inquiries concerning this study or if you have questions or concerns about your rights as a participant in this research, you may contact the above contact persons. A copy of this consent form will be provided for you.

Your signature below is indicated that you consent to volunteer for this study.

Participant	**Witness**	**Investigator**
Name: ----------	Name: ----------	Name: ----------
Sign. ----------	Sign. ----------	Sign. ----------
Date ----------	Date ----------	Date ----------

APPENDIX 2: ETHICAL CLEARANCE

APPENDIX 3: ROW DATA

ID	AGE	HEIGHT	WEIGHT	BMI	grcat	MHG spre	MHG spost	Vj in m	Vjcm post	PaPw post	IHIEndu pre	IHIEndu post	vo2
025	20	1.71	54.50	18.64	1	47.00	56.30	.48	67.00	4480.75	1320	1720	50.85
010	21	1.68	53.50	18.96	1	47.00	58.00	.60	69.00	4556.85	1360	1680	50.51
008	22	1.62	51.10	19.47	1	49.90	57.50	.53	59.00	3841.13	1440	1640	50.18
028	19	1.70	56.50	19.55	1	45.65	58.61	.47	67.00	4571.35	1320	1600	49.84
016	23	1.71	60.20	20.59	1	44.30	57.88	.51	71.00	4981.76	1320	1640	50.18
004	19	1.59	53.30	21.08	1	56.80	58.50	.61	69.00	4547.79	1320	1720	50.85
006	19	1.58	54.30	21.75	1	43.70	54.25	.45	68.00	4532.39	1320	1600	49.84
017	24	1.59	58.00	22.94	1	48.60	55.64	.39	58.00	4093.00	1240	1680	50.51
002	22	1.78	61.10	19.28	2	46.10	53.56	.63	68.00	4840.43	1360	1280	47.15
005	24	1.71	58.30	19.94	2	50.50	55.96	.52	61.00	4288.69	1320	1400	48.16
020	18	1.64	53.70	19.97	2	43.70	51.20	.59	72.00	4748.01	1280	1360	47.82
013	21	1.71	59.10	20.21	2	52.00	58.90	.53	64.00	4507.03	1240	1200	46.48
027	23	1.59	51.90	20.53	2	59.90	58.10	.55	68.00	4423.67	1280	1440	48.50
023	20	1.62	54.00	20.58	2	49.90	50.20	.65	71.00	4700.90	1560	1520	49.17
029	24	1.67	57.50	20.62	2	61.30	59.30	.50	64.00	4434.55	1320	1360	47.82
012	25	1.58	52.80	21.15	2	54.30	57.24	.48	64.00	4221.64	1280	1240	46.82
001	22	1.67	59.20	21.23	2	53.40	57.00	.56	62.00	4390.16	1440	1640	50.18
018	20	1.80	58.50	18.06	3	56.80	56.62	.59	50.00	3901.85	1280	1360	47.82
015	20	1.77	59.20	18.90	3	51.20	50.34	.57	51.00	3948.96	1240	1280	47.15
009	19	1.72	58.10	19.64	3	54.10	54.30	.67	66.00	4719.03	1360	1320	47.49
026	18	1.69	56.30	19.71	3	55.32	55.31	.48	50.00	3530.39	1560	1440	48.50
011	20	1.80	64.50	19.91	3	56.80	57.30	.42	44.00	3537.65	1280	1280	47.15
024	22	1.64	54.40	20.23	3	54.10	54.82	.57	55.00	3747.82	1280	1320	47.49
022	22	1.65	55.80	20.50	3	49.90	50.18	.58	58.00	3993.34	1240	1320	47.49
007	21	1.57	52.00	21.10	3	46.80	51.36	.47	48.00	3214.20	1440	1440	48.50
003	19	1.68	59.90	21.22	3	39.70	41.60	.47	49.00	3632.77	1320	1400	48.16

APPENDEX 4; YO-YO IR1 TEST RECORD SHEET

Yo-Yo Test Recording Sheet

Yo-Yo Intermittent Recovery Test Level 1

Level	5.1						
Level	9.1						
Level	11.1	11.2					
Level	12.1	12.2	12.3				
Level	13.1	13.2	13.3	13.4			
Level	14.1	14.2	14.3	14.4	14.5	14.6	14.7	14.8
Level	15.1	15.2	15.3	15.4	15.5	15.6	15.7	15.8
Level	16.1	16.2	16.3	16.4	16.5	16.6	16.7	16.8
Level	17.1	17.2	17.3	17.4	17.5	17.6	17.7	17.8
Level	18.1	18.2	18.3	18.4	18.5	18.6	18.7	18.8
Level	19.1	19.2	19.3	19.4	19.5	19.6	19.7	19.8
Level	20.1	20.2	20.3	20.4	20.5	20.6	20.7	20.8
Level	21.1	21.2	21.3	21.4	21.5	21.6	21.7	21.8
Level	22.1	22.2	22.3	22.4	22.5	22.6	22.7	22.8
Level	23.1	23.2	23.3	23.4	23.5	23.6	23.7	23.8

Date: _____

Time: _____

Surface: _____

Conditions: _____

For more information, see https://www.thevoyotest.com/

APPENDEX 5; ANOVA AND POST HOC TEST RESULT

ANOVA

maximum hand grip muscular strength post intervation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	99.064	2	49.532	3.908	.035
Within Groups	291.517	23	12.675		
Total	390.582	25			

Multiple Comparisons

Dependent Variable: maximum hand grip muscular strength post intervation

Tukey HSD

(I) group category	(J) group category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
whey protein experimental group	soy protein experimental group	1.36722	1.72992	.713	-2.9651	5.6995
	control group	4.65944*	1.72992	.033	.3271	8.9918
soy protein experimental group	whey protein experimental group	-1.36722	1.72992	.713	-5.6995	2.9651
	control group	3.29222	1.67827	.145	-.9107	7.4952
control group	whey protein experimental group	-4.65944*	1.72992	.033	-8.9918	-.3271
	soy protein experimental group	-3.29222	1.67827	.145	-7.4952	.9107

*. The mean difference is significant at the 0.05 level.

ANOVA

peak an aerobic power output post intervation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2711055.527	2	1355527.763	11.918	.001
Within Groups	2616013.389	23	113739.713		
Total	5327068.915	25			

Multiple Comparisons

Dependent Variable: peak an aerobic power output post intervation

Tukey HSD

(I) group category	(J) group category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
whey protein experimental group	soy protein experimental group	-55.49250	163.87559	.939	-465.8921	354.9071
	control group	647.73750*	163.87559	.002	237.3379	1058.1371
soy protein experimental group	whey protein experimental group	55.49250	163.87559	.939	-354.9071	465.8921
	control group	703.23000*	158.98268	.001	305.0839	1101.3761
control group	whey protein experimental group	-647.73750*	163.87559	.002	-1058.1371	-237.3379
	soy protein experimental group	-703.23000*	158.98268	.001	-1101.3761	-305.0839

*. The mean difference is significant at the 0.05 level.

ANOVA

estimated vo2 max

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.933	2	16.966	27.481	.001
Within Groups	14.200	23	.617		
Total	48.133	25			

Multiple Comparisons

Dependent Variable: estimated vo2 max

Tukey HSD

(I) group category	(J) group category	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
whey protein experimental group	soy protein experimental group	2.33333*	.38180	.001	1.3772	3.2895
	control group	2.59467*	.38180	.001	1.6385	3.5508
soy protein experimental group	whey protein experimental group	-2.33333*	.38180	.001	-3.2895	-1.3772
	control group	.26133	.37040	.763	-.6663	1.1889
control group	whey protein experimental group	-2.59467*	.38180	.001	-3.5508	-1.6385
	soy protein experimental group	-.26133	.37040	.763	-1.1889	.6663

*. The mean difference is significant at the 0.05 level.