COMBINED EFFECT OF PLANT-BASED PROTEIN SUPPLEMENTATION WITH RESISTANCE TRAINING ON MUSCULAR STRENGTH, PROTEIN CATABOLISM, IMMUNE FUNCTIONS AND BONE METABOLISM MARKERS IN ADULT MALES

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COMBINED EFFECT OF PLANT-BASED PROTEIN SUPPLEMENTATION WITH RESISTANCE TRAINING ON MUSCULAR STRENGTH, PROTEIN CATABOLISM, IMMUNE FUNCTIONS AND BONE METABOLISM MARKERS IN ADULT MALES

BY

AZAIZIRAWATI BINTI HJ AHMAD

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AZAIZIRAWATI BINTI HJ AHMAD

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ABSTRAK

Gabungan makanan tambahan protein dan latihan rintangan diketahui dapat membina otot untuk membentuk penampilan fizikal dan meninkatkan kekuatan. Protein berasaskan tumbuhan telah digunakan untuk tahap yang lebih rendah daripada protein berasaskan haiwan, untuk meningkatkan kekuatan otot dan mengurangkan katabolisma protein. Tambahan pula, latihan rintangan telah menunjukkan dapat meningkatkan fungsi imun dan ketumpatan tulang berbanding latihan daya ketahanan. Tujuan kajian ini adalah untuk mengkaji kesan gabungan makanan tambahan protein berasaskan tumbuhan yang terdiri daripada protein soya dan kacang hijau dengan latihan rintangan pada kekuatan otot, katabolisma protein, fungsi imun dan penanda metabolisma tulang pada lelaki dewasa. Dua puluh lapan orang lelaki dewasa yang tidak aktif (Min ± SD umur: 22.72 ± 3.17 tinggi: 169,40 ± 5.76 berat: 68,04 ± 8,97 BMI: 23.70 ± 2.95) telah menawarkan diri secara sukarela dalam ujian sebelum dan selepas 8 minggu kajian intervensi ini. Kekuatan otot telah ditentukan dengan menggunakan dinamometer isokinetik dan prosedur pengambilan darah telah dijalankan untuk analisis penanda-penanda katabolisma protein, fungsi imun dan metabolisma tulang. Peserta dibahagikan secara rawak kepada empat kumpulan iaitu protein gabungan latihan rintangan berasaskan tumbuhan (PBPEX), protein berasaskan tumbuhan sahaja (PBP), latihan rintangan sahaja (EX) atau kawalan (C). Peserta dalam PBPEX dan kumpulan PBP mengambil 16g protein berasaskan tumbuhan yang terdiri daripada protein soya dan kacang hijau manakala

peserta EX dan kumpulan C menggunakan plasebo 16g maltodekstrin. Semua makanan tambahan telah diberikan dalam bentuk serbuk putih dalam uncang yang dimeteri dan dibancuhkan dalam 150ml air kosong. Makanan tambahan diambil setiap hari pada waktu pagi selama 8 minggu. Peserta dalam kumpulan PBPEX dan EX menjalankan latihan rintangan yang terdiri daripada tujuh jenis senaman badan pada 60-70% keamatan satu ulangan maksimum (1-RM) sebanyak tiga set 4-6 ulangan, 3 kali seminggu selama 8 minggu, di bawah seliaan. Semasa hari latihan, peserta dalam kumpulan PBPEX dan EX mengambil makanan tambahan sebaik sahaja sesi latihan tamat. Semua data telah dianalisis dengan menggunakan ujian statistik langkah berulang (ANOVA). PBPEX boleh meningkatkan ($p <0.01$) isokinetik kekuatan parameter puncak akhiran otot fleksi lutut dan bahu dengan lebih signifikan berbanding kumpulan EX. Untuk ukuran katabolisma protein, PBP menunjukkan tahap yang lebih tinggi ($p <0.05$) dalam urea serum dan urea nitrogen darah (BUN) berbanding kumpulan PBPEX, EX dan C masing-masing. Tidak terdapat perbezaan yang signifikan dalam parameter fungsi imun di antara ujian pra dan pasca dalam semua kumpulan. Untuk penanda metabolisma tulang, tiada perubahan ketara secara statistik yang diperhatikan dalam penanda pembentukan dan resorpsi tulang. Gabungan protein berasaskan tumbuhan dan latihan rintangan menunjukkan kesan yang lebih baik untuk meningkatkan kekuatan otot berbanding latihan rintangan sahaja. Bagi penanda katabolisma protein, gabungan protein berasaskan tumbuhan dengan latihan rintangan nampaknya mempunyai potensi untuk mengurangkan peningkatan katabolisma protein yang disebabkan oleh protein berasaskan tumbuhan sahaja. Terdapat kesan yang signifikan terhadap parameter fungsi imun dan penanda metabolisme tulang dengan gabungan protein berasaskan tumbuhan dan latihan rintangan. Gabungan makanan tambahan protein berasaskan tumbuhan dan latihan rintangan memberikan kesan yang lebih baik terhadap kekuatan otot berbanding latihan rintangan sahaja dan makanan tambahan protein berasaskan tumbuhan sahaja. Oleh itu, gabungan protein berasaskan tumbuhan dengan latihan rintangan boleh disyorkan sebagai garis panduan dalam perancangan latihan dan program pemakanan untuk lelaki dewasa tidak aktif.

COMBINED EFFECT OF PLANT BASED PROTEIN SUPPLEMENTATION WITH RESISTANCE TRAINING ON MUSCULAR STRENGTH, PROTEIN CATABOLISM, IMMUNE FUNCTIONS AND BONE METABOLISM MARKERS IN ADULT MALES

ABSTRACT

Combination of protein supplementation and resistance training is known to develop muscle bulk for physical appearances and strength gain. Plant-based protein has been used to a lesser extent than animal-based protein, to increase muscle strength and reduce protein catabolism. Furthermore, resistance training has been shown to enhance immune function and bone density compared to endurance training. The purpose of this study was to investigate the combined effect of plant-based protein supplementation which consists of soy and pea protein with resistance training on muscular strength, protein catabolism, immune functions and bone metabolism markers in adult males.Twenty eight sedentary adults male (Mean±SD age: 22.72 ± 3.17 height: 169.40± 5.76 weight: 68.04 ± 8.97 BMI: 23.70 ± 2.95) volunteered for testing before and after an 8-week of study intervention. Muscular strength was determined by using isokinetic dynamometer and blood taking procedure were performed for protein catabolism, immune function and bone metabolism markers. Participants were randomly assigned into four groups of either plant-based protein combined resistance training (PBPEX), plant-based protein alone (PBP), resistance training alone (EX) or control (C). Participants in PBPEX and PBP groups consumed 16g plant-based protein consists of soy and pea protein while participants in EX and C groups consumed 16g placebo consists of maltodextrin. All supplements were given in the form of white powder in sealed sachets and to be diluted in 150ml of plain water. The supplements were consumed every day in the morning for 8 weeks.

Participants in PBPEX and EX groups performed three sets of 4-6 repetitions, 3 times per week for 8 weeks, of supervised resistance training that consisted of seven types of total body exercises at 60-70% intensity of one repetition maximum (1-RM). During training days, participants in PBPEX and EX groups consumed the supplement immediately after training session. All data were analysed using repeated measures ANOVA. PBPEX could significantly increase ($p<0.01$) more isokinetic muscular strength parameter, i.e knee and shoulder flexion peak torque compared to EX groups. For protein catabolism measures, PBP showed significantly higher level ($p<0.05$) in serum urea and blood urea nitrogen (BUN) than PBPEX, EX and C groups respectively. There were no significant differences in immune function parameters between pre- and post- tests in all the groups. For bone metabolism markers, no statistically significant changes were observed in bone formation and resorption markers. Combination of plant-based protein and resistance training showed greater beneficial effects on enhancing muscular strength than resistance training alone. For protein catabolism, plant-based protein combined with resistance training seems to have potential in attenuating the increase of protein catabolism induced by plant-based protein alone. There were significant effects on immune function parameters and bone metabolism markers with combination of plant-based protein and resistance training. Combination of plant-based protein supplementation and resistance training elicited greater beneficial effects on muscular strength than resistance training alone and plant-based protein supplementation alone. Therefore, combined plant-based protein with resistance training may be recommended as a guideline in planning exercise and nutritional programme for sedentary male adults.

CHAPTER 1

INTRODUCTION

Nowadays, protein supplements are widely used as it is believed that combining the consumption of protein supplements with exercise will promote gains in lean mass and improved physical performance (Erdman *et al.*, 2007; Lieberman, 2010). Protein supplements have been recommended to athletes to enhance nitrogen retention and muscle glycogen resynthesis following exercise, and to prevent protein catabolism during prolonged exercise (Williams *et al*., 2005).

Proteins are organic compounds composed of a sequence of amino acids. It is needed to promote growth and to repair damaged cells and tissues, as well as for a variety of metabolic and hormonal activities. In the body, there are 22 amino acids which can be used to make proteins. Others eight essential amino acids must be acquired from the diet because the body cannot synthesise them (Campbell, 2011). There are two sources of protein that can be found in the diet, which are animal-based protein and plant-based protein. Animal-based protein is much more similar to human body and thus is used more readily than plant-based protein (Campbell, 2011).

Plant-based protein like soy has been used as a protein milk substitute. Soy protein contains a single homogenous fraction whereas milk has two protein fractions known as whey and casein protein. Despite of lacking the amino acid methionine, soy is an excellent source of protein extracted from soybean. Soy protein concentrate contains 70% protein, and whereas soy protein isolate contains 90% protein. Soy protein also contains isoflavone glycosides which is beneficial for health (Richard, 2011). According to Segounis (2004), soy protein is extracted from the annual leguminous soybean plant that has been found in the food chain for over 5,000 years, it is the only plant-based protein considered to be a high-quality protein, containing all of the essential amino acids in the ratios needed to support growth and development. The soybean was introduced to the United States in the 1880s, and before then was, and still is a staple of the Asian diet. Pea (*Pisum sativum*) is another source of plant-based protein, containing 85% of proteins and particularly rich in essential branched-chain amino acids (BCAA; leucine, isoleucine and valine). It is known to play an important role in muscle protein synthesis (Babault *et al*., 2015).

Plant based protein from soy had been reported of its benefits to health and performance, as it is known to provide an excellent source for protein including likely result of reduction in the intake of saturated fat and cholesterol compared to animal protein (Hoffman & Falvo, 2004). Soy protein is beneficial to cardiovascular and overall health due to its high content of polyunsaturated fats, fibre, vitamins, minerals and low content of saturated fat (Sacks *et al*., 2006).

Nowadays, resistance training exercise has gained recognition in term of the physiological adaptation and health benefits that occur related with the exercise (Phillips, 2016). Resistance training is known as a mean for developing and maintaining muscle strength, endurance, power and muscle mass (Brown, 2007), hence it relationship to health factors and chronic disease has been recognised. Resistance training has been shown to reduce factors associated with coronary heart disease, diabetes and osteoporosis (Warburton *et al*,. 2006).

It is generally known that, combination of protein supplementation and resistance training can develop muscle bulk for physical appearances and gain strength. The American Heart Association recognised physical training as a way to improve overall health and fitness. The used of soy protein as based of supplementation has been suggested in reducing the risk of cardiovascular disease by improving lipid profiles and the insulin-to-glucagon ration and lowers oxidative stress (DeNysschen *et al.*, 2009). However, there are still limited studies to show whether soy will support the skeletal muscle protein accretion in response to resistance training as effective as animal- based protein.

To our knowledge, to date information on the effect of combined plant-based protein supplementation consists of soy and pea protein with resistance training on muscular strength, protein catabolism, immune functions and bone metabolism markers in sedentary adult males.

1.1 Problem statement

To our knowledge, to date there are no studies investigated the effect of combined plant-based protein supplementation consists of soy and pea protein with resistance training on isokinetic muscular strength, protein catabolism, immune functions and bone metabolism markers in sedentary adult males. Therefore, the present study was proposed.

1.2 Objectives of study

1.2.1 General objective*:*

To investigate the combined effect of plant-based protein supplementation consists of soy and pea protein with resistance training on muscular strength, protein catabolism, immune functions and bone metabolism markers in adult males.

1.2.2 Specific Objectives*:*

i. To investigate the combined effect of plant-based protein supplementation with resistance training on isokinetic muscular peak torque in adult males.

ii. To investigate the combined effect of plant-based protein supplementation with resistance training on blood protein catabolism markers (serum creatinine, urea and blood urea nitrogen (BUN)) in adult males.

iii. To investigate the combined effect of plant-based protein supplementation with resistance training on blood immune function markers of white blood cell (WBC), lymphocytes, neutrophil, monocytes, eosinophil basophil counts and immunophenotyping (T cell, T helper cell, T cytotoxic cell, B cell and natural killer cells) in adult males.

iv. To investigate the combined effect of plant-based protein supplementation with resistance training on blood bone metabolism markers (serum alkaline phosphatase (ALP) for bone formation marker and cross linked C-telopeptide of type 1 collagen (CTX 1) for bone resorption marker) in adult males.

1.3 Significance of study

It is hoped that the findings obtained from this study can be used to formulate guidelines in planning training or exercise and nutritional programmes for increasing muscular strength, immune function and bone health among sedentary male adult population.

1.4 Hypotheses of the study

I. H_{O1}: There are no significant differences in muscular strength among male adults of combined plant-based protein supplementation with resistance training group compared to plant-based protein supplementation alone, resistance training alone and control groups.

   H_{A1}: There are significant differences in muscular strength among male adults of combined plant-based protein supplementation with resistance training group compared to plant-based protein supplementation alone, resistance training alone and control groups.

II. H_{O2}: There are no significant differences in protein catabolism among male adults of combined plant-based protein supplementation with resistance training group compared to plant-based protein supplementation alone, resistance training alone and control groups.

   H_{A2}: There are significant differences in protein catabolism among male adults of combined plant-based protein supplementation with resistance training programme group compared to plant-based protein supplementation alone, resistance exercise alone and control groups.

III. H_{O3}: There are no significant differences in immune function among male adults of combined plant-based protein supplementation with resistance training group compared to plant-based protein supplementation alone, resistance training alone and control groups.

   H_{A3}: There are significant differences in immune function among male adults of combined plant-based protein supplementation with resistance training programme group compared to plant-based protein supplementation alone, resistance exercise alone and control groups.

IV. H_{O4}: There are no significant differences in bone metabolism markers among male adults of combined plant-based protein supplementation with resistance training group compared to plant-based protein supplementation alone, resistance training alone and control groups.

H_{A4}: There are significant differences in bone metabolism markers among male adults of combined plant-based protein supplementation with resistance training programme group compared to plant-based protein supplementation alone, resistance exercise alone and control groups.

CHAPTER 2

LITERATURE REVIEW

2.1 Resistance training exercise

Resistance training exercise has gained popularity over the last decade as it is known to have numerous benefits to offer. This type of exercise causes the muscles to contract against an external resistance with the expectation of increases in strength, tone, mass, and also endurance (Granacher *et al*,.2016). The effectiveness of a resistance training programme is dependent upon several factors including frequency, volume of training (sets × repetitions × resistance), and mode of training i.e., free weight, multiple resistance weight machine, resistance band or free body weight (Brown, 2001).

According to Mangine *et al* (2015), the most important factor for developing muscle strength is the intensity of resistance training, whereas the total training volume (sets x repetitions x resistance) is more important for the development of muscular endurance and muscle mass. Brown (2001) suggested that three sets of 6-12 repetitions performed for three days per-week is a typical exercise that been prescribed for many resistance training programmes. Moderate intensity programmes are usually recommended for the most nonathletic adult populations including programmes designed for adult fitness, health maintenance, and orthopedic rehabilitation (Roxburgh *et al*, 2014).

2.2 Exercise, protein supplementation and muscular strength

Resistance training exercise has gained the popularity over the last decade as it is known to have numerous benefits to offer. This type of exercise causes the muscles to contract against an external resistance with the expectation of increases in strength, tone, mass, and also endurance. Many studies have been done to prove that resistance training has well-established positive effects on muscular strength and muscle growth. Aiming for greater gains in lean mass and muscle strength are essential as the duration and frequency of resistance training increases. Besides, many trained individuals consumed protein supplement to achieve this particular aim. Bianco *et al* (2001) reported that 30.1% of male athletes use dietary supplements during training as a way to gain muscle and strength. The authors also mention that whey protein shakes (50.0%) supplemented with creatine and amino acids (48.3%) were the most frequent choices amongst users.

Muscle strength is defined as maximum force that can be generated by muscle group. The measurement of muscular strength is a common practice in the evaluation of the effectiveness of training programs in football players, shot putters, weight lifters and other power athletes (Granacher *et al*., 2016). Meta-analysis of 20 randomised control trials among healthy male young adults showed that muscle strength increased significantly after high eccentric resistance training compared to concentric exercise (Roig *et al*., 2009). There were also improvements in motor unit potential, as well as muscle strength and endurance in healthy male college students after 8 weeks of resistance training in a research conducted by Ae Rim, (2014).

Protein supplementation during resistance exercise training was reported to augment hypertrophic gains. Protein ingestion and the resultant hyper aminoacidemia provides the building blocks (indispensable amino acids – IAA), triggers an increase in muscle protein synthesis (MPS), suppression of muscle protein breakdown (MPB) and net positive protein balance (Phillips, 2004).

According to a review on the effects of protein supplement complimented with resistance training on muscle mass, strength, aerobic and anaerobic power, it was found that there are existing evidences that support the rationale for including protein supplement before or immediately following a bout of resistance or aerobic exercise to enhance protein synthesis and anabolic signalling (Pasiakos *et al.*, 2015).

As for the effect of plant-based protein combining resistance training on muscle strength, a study showed no significant differences in muscle thickness and muscle strength between pea and whey protein with 12 weeks resistance training among 161 young healthy male adults (Babault *et al.*, 2015). In another study, the result showed increases of muscle mass and strength and lower percentage of body fat after 9 months of resistance training with soy isoflavone consumption among postmenopausal woman (Orsatti *et al*., 2017).

Tang et al. (2009) concluded in their study that the feeding-induced simulation of mixed MPS in young men is greater after whey hydrolysate or soy protein consumption than casein both at rest and after resistance exercise. Moreover, despite both being fastest absorbing proteins, whey hydrolysate stimulated MPS to a greater degree than soy after resistance exercise.

According to Tipton et al (2001), the muscle protein synthesis is dependent on the availability of the protein after ingestion to offset the negative muscle protein balance (catabolism) caused by resistance training.

2.3 Exercise, protein supplementation and protein catabolism

Protein catabolism is the process of protein breakdown into amino acid to the cell through the plasma membrane. Protein catabolism causes the release of amino acids from endogenous proteins, when amino acid is metabolised, urea is the major nitrogen end-product (Hutchinson *et al.*, 2011). Urea is the primary metabolite derived from dietary protein and tissue protein turnover whereas creatinine is the product of muscle creatine catabolism. The rate of creatinine appearance in blood is an indicator of endogenous protein catabolism. Absolute creatinine production declines with age in line with decreasing muscle mass (Hosten, 1990)

In a study on the influence of muscle mass and physical activity on serum and urinary creatinine, it was found that both serum and urinary creatinine were significantly correlated with body weight, but the level of correlation with lean mass was even greater (Baxmann *et al.*, 2007). In the same study, individuals with moderate or intense physical activity presented higher urinary creatinine, probably as a result of the larger muscle mass and the higher mean protein intake.

In a study which examined the effect of branched-chain amino acid (BCAA) on serum indicator of muscle damage after prolonged exercise, the result showed that BCAA supplementation significantly reduced lactase dehydrogenase and creatinine kinase after 120 minutes cycling at intensity of 70% of maximal oxygen consumption (VO_2max) (Coombes & McNaughton, 2000).

2.4 Exercise, protein supplementation and immune function

Exercise is also known to have an effect on immune function. Human immune system protects the body from diseases or infections by a pathogenic organism through the development of an antibody or cell mediated antibody (Taber's 2001). Moderate exercise has been linked to positive immune system response and a temporary boost in the production of macrophages. Based on Quinn 2008, immune cells circulate through the body more quickly and efficiently to kill pathogen during the moderate exercise.

In resistance training related to immune function, there were no differences in white blood cell, haemoglobin, haematocrit, percentages of monocytes and granulocytes when comparing between resistance exercise and intermittent intense aerobic exercise in trained middle aged women population (Cardoso *et al.,* 2012).

In another study on the acute immune response to exhaustive resistance exercise, the results demonstrated that leg squat exercise to exhaustion resulted in very similar immune responses associated with intense endurance exercise, as leukocytosis, lymphocytosis and lymphocytopenia reported similar with the previous study on high intensity cardiorespiratory exercise (Nieman *et.al.*1999).

Besides exercise, nutritional supplementations also play an important role in boosting immune function. Increasing evidence showed that dietary supplementation of specific amino acids in humans with malnutrition and infectious disease enhances the immune status by regulating the immune. These regulations includes 1) the activation of T lymphocytes, B lymphocytes, natural killer cells and macrophages; 2) the signalling of cellular redox state, gene expression and lymphocyte proliferation and 3) the production of antibodies, cytokines and other cytotoxic substances (Li *et al.*, 2007).

It was reported that BCAA supplementation before and after exercise could decrease exercise-induced muscle damage and promote muscle protein synthesis. BCAA also modified the pattern of exercise-related cytokine production, leading to a diversion of the T lymphocyte (adaptive immunity) response towards T helper type 1 immune cell. Thus, BCAA is believed to be beneficial for muscle recovery and immune regulation (Negro *et al*., 2008).

2.5 Exercise, protein supplementation and bone metabolism

Changes in muscle mass and strength can affect bone in mass, size, and strength correspondingly, as to support the concept of the relationship between a functional muscle and bone unit (Daly *et al*., 2004). Bone is a metabolically active tissue that undergoes continuous remodelling by two counteracting processes, namely bone formation and bone resorption. These processes rely on the activity of osteoclasts knows as resorption, osteoblasts as formation and osteocytes as maintenance (Fogelman *et al.*, 2012).

Fujimura *et al* (1997) reported that there was an increase in marker of bone formation and suppression on bone resorption in a study conducted on the effect of 4 month resistance training among young male adult of age 23-31 years old. Their findings were not consistent with a study conducted by Mullins and Sinning (2005), in which they reported progressive resistance training did not increase bone formation marker concentrations, with serum bone specific alkaline phosphate declining (BAP) and serum osteocalcin (OC) showing no change after 12 weeks of resistance training combined with 2.4g/kg body weight/day of protein intake among eumenorrheic women of age 18-30 years old.

CHAPTER 3

METHODOLOGY

3.1 Sample size calculation

The sample size calculation was based on primary outcome measure of 1-RM bench press strength result from study by Burke *et al* (2001), anticipating a significant change of 7% or greater in protein-supplemented group combined with strength training. The standard deviations of the group were 7.85 (Burke et al., 2001). The power of the study was set at 90% confidence interval, 80% power and alpha 0.05 with an effect size of 0.7 (Cohen, 1998). Calculated sample size was equal to 7 participants per group (total 28). F-test and ANOVA repeated measures between factors were used for the sample size calculation using G* Power software 3.1.9.2 as shown in Figure 3.1.

F tests – ANOVA: Repeated measures, between factors

Analysis:	A priori: Compute required sample size		
Input:	Effect size f(V)	=	0.6934154
	α err prob	=	0.05
	Power (1−β err prob)	=	0.80
	Number of groups	=	4
	Number of measurements	=	2
Output:	Noncentrality parameter λ	=	13.4630977
	Critical F	=	3.0087866
	Numerator df	=	3.0000000
	Denominator df	=	24.0000000
	Total sample size	=	28
	Actual power	=	0.8200828

Figure 3.1 Sample size calculations

3.2 Participant recruitment

Approved for the study was obtained from the Research ethics committee of Universiti Sains Malaysia (USM) (Appendix A)**.**Twenty eight (28) Malaysian male adults were recruited as participants in this study, however only twenty five (25) participants were able to complete the protocol. Three (3) participants discontinued the study due to unable to commit with the protocol and personnel matter.

The male participants of the study, aged from 18-30 years old, were recruited among the population in the area of Kota Bharu. Recruitment advertisement via poster and flyers were given by hand, posted in social media and at the notice board of gym fitness centre and at the student campus around Universiti Sains Malaysia (USM) (appendix B). All participants were informed about the nature and possible risks associated with the experimental procedure; prior written informed consent was obtained from the participants.

3.3 Inclusion criteria

All participants were healthy volunteers who were engaged in exercise for less than 2 times a week with normal body mass index (BMI). Participants were free from lower-extremity injuries or dysfunction for the past one year and not taking any type of supplementations and not engage with other training programme.

3.4 Exclusion criteria

Participants were assessed for health risks and suitability by completing the Physical Activity Readiness Questionnaire (PAR-Q)(CSEP, 2011) (Appendix C). Participants with severe knee pain restricting exercise activity and movement, congenital disorder and other ligamentous injury to the knee, heart complications or any other forms of diseases were excluded. Participants were also excluded if they had taken anabolic steroids or other anabolic agents known to increase performance during the training period. Participants who had allergy to any of the ingredients in the experimental supplements were excluded.

3.5 Study location

The resistance training programme were conducted at a local gymnasium situated at Taman Sri Kota, Kg Pasir Pekan, Wakaf Bharu, 16250 Tumpat, Kelantan, and data collection were carried out at Exercise and Sport Science laboratory, Health Campus USM, Kubang Kerian, Kelantan.

During the training sessions, all the participants were supervised by experienced trainers. Certified health care provider was on stand-by to monitor participants' safety throughout the training session.

3.6 Method of sampling and randomisation

Simple random sampling was carried out in this study. Each participant was chosen entirely from the population in the Kota Bharu and they had an equal chance of being included in the sample.

Randomisation of participants into the groups was done using Research Randomizer by Urbaniak and Plous (2013). It is a computer-based "pseudo-random number generator" as the numbers were generated by use of complex algorithm that gives appearance of randomness. Each of the participants was assigned by a unique number (representing the number of participants) to generate the sample group.

Participants were assigned into four groups as follows:

1. Sedentary with placebo control group (C)
2. Sedentary with plant-based protein supplement group (PBP)
3. Resistance exercise with placebo group (EX)
4. Resistance exercise with plant-based protein supplement group (PBPEX)

3.7 Plant-based protein supplementation

Participant in both PBP and PBPEX group consumed 16 g of plant-based protein powder (appendix D) mixed with 150 ml of plain water per day after breakfast, 7 days per week for 8 weeks, except on the exercised days, participants in the PBPEX group were required to ingest plant-based protein drink 30 minutes after completion of training. The protein supplement contains 9.8 g of combined isolated soy protein and pea protein which has been certified Halal (appendix E). Placebo (16 g) applied in this study contains Maltodextrin DE10-12 which has been widely used and approved as a safe food additive by the U.S Food and Drug Administration. The placebo was mixed with 150 ml of plain water and consumed by participants in group C and EX every day after breakfast, 7 days per week for 8 weeks. For EX group, the placebo was consumed immediately post workout session during the training day.

3.8 Double-blind method

An individual (lab technician officer from the sports science laboratory, Health Campus, USM) who was not participating in data analysis of the study was assigned to prepare the supplementation and randomise the participants into 4 groups. Supplement and placebo were distributed in powder form in an unmarked sachet for 8 weeks supply per participant in the PBP and PBPEX groups for supplement, and EX and C groups for placebo. The lab technician filled the unmarked sachet. Each supplement was prepared as dry powder in sealed sachets and with a number code to ensure study blinding. The blinding of participants and researchers were assured. The participants recorded their daily intake of supplementation or placebo in the provided food diary (appendix F). Participants were also recorded their baseline dietary intake habits and food intake information in the same provided food diary.

Anthropometric and body composition, muscular strength, and haematological pre-test measurements for protein catabolism, immune function and bone metabolism were conducted prior to the start of the study. The post-test measurements were conducted 8 weeks after intervention. The flow chart of the experimental design of the study is shown in Figure 3.2.

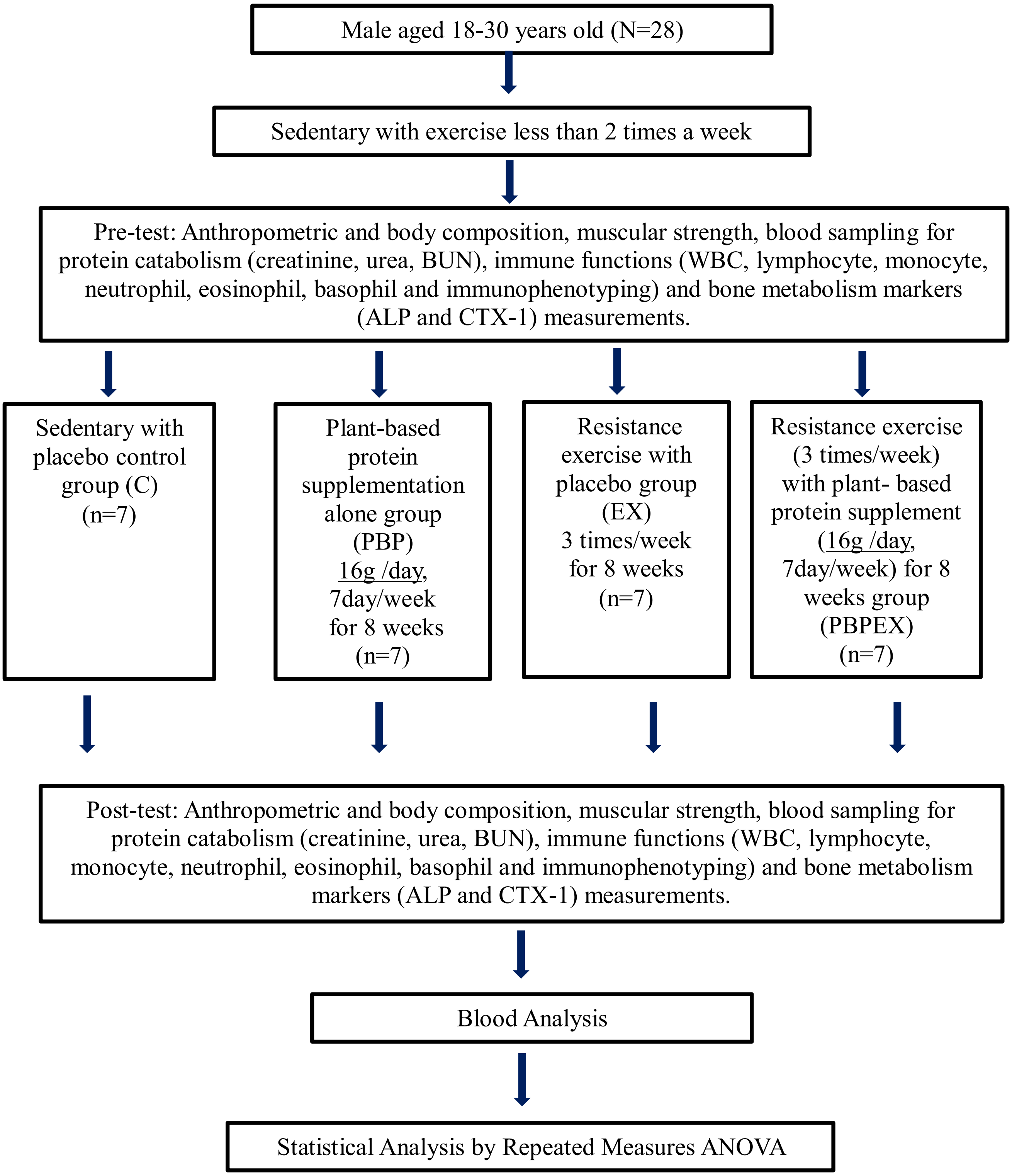

Figure 3.2 Flow chart of the experiment design

3.9 Anthropometry and body composition analysis

At pre- and post-tests, participants' body height and weight were measured while wearing light clothing without shoes. Body height was determined to the nearest 0.1 cm using a stadiometer (SECA, UK). Body weight (BW) and body composition analysis was conducted using body composition Analyser (TANITA, TBF-410 model, Japan) and were determined to the nearest 0.1 kg and 0.1% using an electronic scale. The measured parameters were body mass (kg), fat free mass (kg) and fat mass (kg). Prior to measurements, participants were instructed to remove all objects attached to the body.

3.10 Isokinetic Muscular Strength Test

Participants were undergoing a familiarisation trial before the actual muscular strength test on a separate day. During the familiarisation, the participant was exposed on demonstration of technique using Biodex Isokinetic dynamometer (Biodex System 3 Pro, New York, USA) to measure upper and lower limb peak torque (strength).

At pre- and post-tests, participants were tested using the similar Biodex Isokinetic dynamometer machine for shoulder and knee peak torque. Two different angular velocities were used ($60^{\circ}.s^{-1}$ and $180^{\circ}.s^{-1}$) to measure shoulder and knee flexion and extension muscular peak torque. The test procedure was briefed to all the participants and all participants were requested to do stretching exercise for one minute before performing the test. Participants were required to do 5 repetitions for the $60^{\circ}.s^{-1}$ and 10 repetitions for the $180^{\circ}.s^{-1}$ angular velocities, and they were given 60s to rest between each angular velocity. Verbal encouragement was consistently provided during all attempts. Essentially, all participants were ensured that they did not experience any joint pain due to testing procedures. They were monitored for safety throughout the procedures.

3.10.1 Knee extension/flexion testing protocol

The participant was seated while leaning against a backrest inclined at 16^0 from the vertical and 6° from horizontal. The chair and the dynamometer were rotated to 90°. The axis of knee was aligned with the axis of Biodex dynamometer exercise arm. The dynamometer was slid along travel position to the lateral side of the leg to be tested. Knee attachment was adjusted so that it was proximal to medial malleoli. The participant's seated position was secured firmly using pelvic, torso and thigh straps in order to minimise body movement during the test. Shoulder straps were applied diagonally across the chest with the participant's arms cross and their palm rested on shoulders to attenuate excessive upper body movement and muscular substitution. The lateral femoral epicondyle was used as a bony landmark for matching the axis rotation of the knee joint and the axis rotation of the dynamometer shaft. The resistance pad was positioned at a two- finger-width distance above the malleoli on the lower leg. The range of motion of knee extension and flexion was set at 90°.

Knee extension and flexion peak torque were tested at speeds of $60^{\circ}.s^{-1}$ and $180^{\circ}.s^{-1}$ for muscular strength. The speeds were chosen due to their predominance in previously published isokinetic studies (Brown et al., 2007). Participants completed 5 repetitions for the $60^{\circ}.s^{-1}$ angular velocity and 10 repetitions for the $180^{\circ}.s^{-1}$ angular velocity for both legs. Sixty second of rest was given to the participants between each angular velocity.

3.10.2 Shoulder extension/flexion testing protocol

Measurements of shoulder extension and flexion muscular strength were carried out with the participants in a sitting position. The participants were stabilised with shoulder, waist and tight straps. The dynamometer was rotated to 0° and the seat was rotated to 15°. The axis of dynamometer was set aligned to the glenohumeral flexion and extension axis. The participants performed the test with their arm held with the elbow extended, hands pronated to grip the dynamometer side handles. The length of the dynamometer's lever arm was adjusted for each participant to achieve an optimal position with an extension of the elbow joint.

Participants were instructed to do 5 repetitions of shoulder flexion and extension at $60^{\circ}.s^{-1}$ angular velocity and 10 repetitions for the $180^{\circ}.s^{-1}$ angular velocity for both shoulders. ten second of rest was given to the participants between each angular velocity. Participants were verbally encouraged to perform the test at their best.

3.11 Resistance Training Protocol

Participants in both EX and PBPEX groups performed resistance training exercises and managed to attend at least 85% of the training programme. The setting of the intensity of resistance training was based on the percentage of one repetition maximum (1-RM) (Gail & Künzell, 2014), it was predicted based on the number of times the weight could be lifted by the participants.

All exercises of the resistance training were performed in 3 sets with 4-6 repetition at 60-70% of intensity from prediction 1-RM, with 2-5 minutes of resting period between each sets, based on a modified protocol recommended for beginners by National Strength and Conditioning Association (NSCA)(Brown, 2007). The rate of repetitions was carried out in a controlled fashion, with a concentric action of approximately one second and an eccentric action of approximately two seconds. Frequency of the training program was three sessions per week with at least 48 h of recovery between sessions, as recommended for beginners (Ratamess *et al* 2009). The intensity for a given exercise was increased to 5% whenever participant could perform more than the prescribed number of repetitions of a particular exercise. Twenty four sessions were performed during the 8 weeks training period. Each participant was required to spend at least 45-60 minutes for each resistance training session. During the exercise sessions, participants were verbally encouraged to perform all complete sets. Training table is attached in appendix G.

The resistance training protocol consisted of seven stations with each station targeting training of major muscle groups of the body. The seven stations were flat barbell press, machine shoulder press, wide grip lateral pull-down, seated cable row, barbell back squat, machine leg press, and machine leg extension. Participants were instructed to refrain from performing any resistance type of training or high intensity anaerobic training other than the training protocol prescribed during the duration of the study.

Before starting, the participants were required to carry out cardiovascular warm up session for 10-15 minutes using either the treadmill or ergo cyclometer. Static stretching prior to exercise was not recommended as it has been shown to be detrimental to dynamometer-measured muscle strength (Page, 2012).

Figure 3.3 Flat barbell press

The first station was flat barbell press (please refer to Figure 3.3) training of pectoralis, anterior deltoid and triceps brachii muscles. Participants were required to lay flat on the bench and ensured the both feet made contact with the floor and hips, upper back and the head touches the bench. Participants held the bar with a grip slightly outside the shoulder width and with the thumb wrapping around the bar. The bar was lowered in a controlled motion with the movement of the bar arcing until lightly touched the lower portion of the chest. The upper arm angle was maintained approximately 45º from the torso before the barbell was lifted up to the starting position by using same path and body position. A gym instructor was on standby for spotting by removing the bar from the rack and supporting the bar over the shoulder.

Figure 3.4 Machine shoulder press

The second station was machine shoulder press (please refer to Figure 3.4) training of anterior and medial deltoids, and triceps brachii muscles. Participants were required to adjust the seat height so that the handles were aligned with shoulder height. The set handle was gripped with the palm facing toward each other. The body was positioned with the chest up and the shoulders and head back against the back pad. The arms were then fully extended in a slow and controlled motion. The handles were returned in starting position without letting the resistance rest on the weight stack between repetitions.