EFFECTS OF COMBINED *CHANNA STRIATUS* SUPPLEMENTATION WITH COUNTERMOVEMENT JUMPING EXERCISE ON BONE PARAMETERS, MUSCULAR PERFORMANCE AND ANTIOXIDANT STATUS IN YOUNG PHYSICALLY INACTIVE MALES

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by

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LIST OF SYMBOLS

AA	Arachidonic acid
ALA	α-linoleic acid
BALP	Bone alkaline phosphatase
BCAA	Branched-chain amino acids
BMD	Bone mineral density
BMU	Basic multicellular unit
BPAQ	Bone physical activity questionnaire
CTX-1	C-telopeptide of type 1 collagen
DHA	Docosahexaenoic acid
DHEA	Dehydroepiandrostone
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
DPD	Deoxypyridionoline
DXA	Dual X-ray absorptiometry
ELISA	Enzyme-linked immunosorbent assays
EPA	Eicosapentaenoic acid
ER	Estrogen receptor
HRP	Horseradish peroxidase
LA	Linoleic acid
LSCS	Post lower segment caesarean section women
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
NTX	N-terminal telopeptide of type 1 collagen
OC	Osteocalcin
PSS	Patient satisfaction score

PUFA	Polyunsaturated fatty acids
PYD	Pyridinoline
P1NP	Procollagen type 1 N-propeptide
QCT	Quantitative computed tomography
RANK	Receptor activator of nuclear factor κβ
RANKL	Receptor activator of nuclear factor κβ by its ligand
ROS	Reactive oxygen species
SOS	Speed of sound
SPSS	Statistical Package for Social Science
VAS	Visual Analogue Scale Scores
VACS	Visual Analogue Cosmetic Scale
VAPS	Visual Analogue Pain Score
VSS	Vancouver Scar Scale
1CTP	Carboxyterminal telopeptide of type 1 collagen
-SH	Sulfhydryl groups

LIST OF ABBREVIATIONS

[a]	Significantly different from pre-test ($p < 0.05$)
[b]	Significantly different from control group ($p < 0.05$)
E_2	Estradiol
T	Testosterone
α	Alpha
β	Beta

LIST OF APPENDICES

KESAN GABUNGAN SUPLEMENTASI *CHANNA STRIATUS* DENGAN SENAMAN LOMPATAN BERPANTUL TERHADAP PARAMETER TULANG, PRESTASI OTOT DAN STATUS ANTIOKSIDA DALAM KALANGAN LELAKI MUDA TIDAK AKTIF SECARA FIZIKAL

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji kesan gabungan supplemen *Channa Striatus* dengan senaman lompatan berpantul selama 12 minggu terhadap parameter tulang, prestasi otot dan status antioksida. Kajian berbentuk kawalan secara rawak yang selari telah dijalankan dalam kalangan lelaki muda tidak aktif selama 12 minggu tempoh intervensi. Tiga puluh lapan orang lelaki muda yang sihat dan tidak aktif berumur 21.7 ± 1.44 tahun telah dipilih dan dibahagikan secara rawak kepada empat kumpulan: kumpulan kontrol (C) (n=9), kumpulan supplemen *Channa Striatus* (CS) (n=9), kumpulan senaman lompatan berpantul (J) (n=10) dan kumpulan gabungan senaman lompatan dengan supplemen *Channa Striatus* (CSJ) (n=10). Peserta-peserta dalam kumpulan J dan CSJ menjalani senaman lompatan berpantul sebanyak tiga kali seminggu selama 12 minggu yang terdiri daripada dua sesi pada setiap hari diselangi dengan 6 jam tempoh berehat di antara dua sesi berkenaan dalam sehari. Peserta dalam kumpulan CS dan CSJ mengambil 1 biji *Channa Striatus* (500mg) pada waktu pagi setiap hari selama 12 minggu. Parameter-parameter yang telah diukur pada pra dan pasca ujian termasuklah komposisi badan, kelajuan bunyi tulang (penanda ketumpatan mineral tulang), kuasa dan daya lompatan, puncak tork (kekuatan) dan kuasa otot isokinetik, kepekatan penanda perolehan tulang darah, penanda tekanan oksidatif dan hormon testosteron darah. Terdapat peningkatan signifikan ($p < 0.05$) kelajuan bunyi

tulang pada tulang '*midshaft tibia*' dalam kumpulan CS, J dan CSJ pada pasca ujian. Selain itu, kumpulan CS menunjukkan penurunan signifikan secara statistik ($p < 0.05$) dalam serum alkali fosfatase tulang (BALP) selepas 12 minggu tempoh intervensi. Nilai lebih besar yang signifikan secara statistik dapat diperhatikan dalam kumpulan J dan CSJ pada kaki dominan dan bukan dominan dalam puncak tork dan kuasa otot isokinetik pada halaju sudut $60^{o}.s^{-1}$ and $300^{o}.s^{-1}$ apabila dibandingkan dengan kumpulan C. Tambahan pula, nilai signifikan ($p < 0.05$) yang tinggi diperolehi dalam kumpulan CS pada kaki dominan dan bukan dominan dalam puncak tork dan kuasa otot isokinetik pada halaju sudut $300^{o}.s^{-1}$ berbanding dengan kumpulan C. Kepekatan serum protein karbonil dalam kumpulan J dan CS meningkat secara signifikan ($p < 0.05$) pada pasca ujian. Pada akhir kajian, kepekatan serum malondialdehyde menunjukan penurunan yang signifikan ($p < 0.01$) dalam kumpulan CS, J dan CSJ. Kepekatan serum testosteron menunjukkan penurunan yang signifikan ($p < 0.05$) dalam kumpulan CS dan CSJ selepas intervensi. Seterusnya, kepekatan serum albumin meningkat secara signifikan ($p < 0.05$) dalam kumpulan CSJ pada pasca ujian. Hasil kajian ini menunjukkan bahawa penggabungan pengambilan supplemen *Channa Striatus* dan senaman lompatan berpantul (CSJ), senaman lompatan berpantul sahaja (J) dan supplemen *Channa Striatus* sahaja (CS) untuk 12 minggu memberikan kesan bermanfaat pada status kesihatan tulang, kekuatan dan kuasa otot dan meningkatkan status antioksida berbanding dengan kumpulan kontrol (C) secara amnya. Justeru itu, penggabungan senaman lompatan berpantul dan pengambilan supplemen *Channa Striatus* berpotensi untuk disarankan bagi meningkatkan status kesihatan tulang, prestasi otot dan status antioksida.

EFFECTS OF COMBINED *CHANNA STRIATUS* SUPPLEMENTATION WITH COUNTERMOVEMENT JUMPING EXERCISE ON BONE PARAMETERS, MUSCULAR PERFORMANCE AND ANTIOXIDANT STATUS IN YOUNG PHYSICALLY INACTIVE MALES

ABSTRACT

The purpose of this study was to investigate the combined effects of *Channa Striatus* supplementation with countermovement jumping exercise for 12 weeks on bone parameters, muscular performance, and antioxidant status. A parallel randomised controlled study was conducted among young inactive males for a 12-weeks intervention period. Thirty-eight healthy physically inactive young males aged 21.7 ± 1.44 years old were recruited and randomised into four groups: control group (C)(n=9), *Channa Striatus* supplementation group (CS) (n=9), jumping exercise group (J) (n=10) and combined jumping exercise with *Channa Striatus* supplementation group (CSJ) (n=10). Participants in J and CSJ groups performed countermovement jumping exercise 3 times per week for 12 weeks which consisted of 2 sessions per day with 6 hours resting period between the sessions in each day. Participants in CS and CSJ groups consumed 1 tablet of *Channa Striatus* (500mg) in the morning every day for 12 weeks. Parameters measured at pre- and post-tests included body composition, bone speed of sound (indicator of bone mineral density), jumping power and force, isokinetic muscular peak torque (strength) and power, blood bone turnover markers, blood oxidative stress markers and testosterone concentrations. There were significant increase ($p < 0.05$) of bone speed of sound midshaft tibia in CS, J and CSJ groups at

post-test. Besides, CS group showed statistically significant decreased ($p < 0.05$) in serum bone alkaline phosphatase (BALP) after 12 weeks of intervention period. Statistically significant greater values ($p < 0.05$) were observed in J and CSJ groups in dominant and non-dominant leg isokinetic muscular peak torque and power at $60^{o}.s^{-1}$ and $300^{o}.s^{-1}$ angular velocities compared to C group. In addition, there was statistically significant higher value ($p < 0.05$) in CS group in dominant and non-dominant leg of isokinetic muscular peak torque and power at $300^{o}.s^{-1}$ angular velocity compared to C group. Serum protein carbonyl concentration in J and CS groups increased significantly ($p < 0.05$) at post intervention. CS, J and CSJ groups had significant decreased of serum malondialdehyde concentration ($p < 0.01$) at the end of the study. There were significant decreases of serum testosterone concentration ($p < 0.05$) in CS and CSJ groups after intervention. Next, serum albumin concentration was significantly increased ($p < 0.05$) in CSJ group at post-test. These study findings imply that combined *Channa Striatus* supplement and countermovement jumping exercise (CSJ), jumping exercise alone (J) and *Channa Striatus* supplementation alone (CS) for 12 weeks elicited greater beneficial effects on bone health status, muscular strength and power and improved antioxidant status compared to control groups (C) generally. Hence, combining countermovement jumping exercise and *Channa Striatus* supplementation has potential to be recommended for enhancing bone health status, muscular performance, and antioxidant status.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

The prevalence of low bone mineral density (BMD) has been reported to be high among inactive individuals compared to physically active young men (aged 18-24 years old) (Chin et al., 2012b). It is generally known that bone strength is associated with physical activity. Exercise could produce force and mechanical loading which enable to strengthen the bone in active individuals (MacKelvie et al., 2002). This was in line with Boot et al. (2010) which stated that mechanical loads especially muscle force helps in regulating bone strength. Physical activities that are dynamic, moderate to high magnitude and short duration give beneficial effects on bone (Abedi et al., 2012; Geraldes et al., 2016; Ju et al., 2014; Judex & Rubin, 2010). Load magnitude produced via impact with an object such as racquet sports and impact with the ground such as jumping (Weaver et al., 2016). Other than that, muscle force during the lift phase in jumping and vaulting and during resistance exercise produced an osteogenic load (Baptista et al., 2016; Janz et al., 2015). Meanwhile, Janz and Baptista (2018) mentioned that rest interval periods in physical activities is also essential for osteogenic effects.

Jumping exercise is a type of dynamic high impact loading activity compared to other types of exercise and it has been shown to be more beneficial for bone health. This has been reported in both animal models (Ju et al., 2020; Ooi et al., 2014;

Tavafzadeh et al., 2011) and also human studies (Erickson & Vukovich, 2010; Hinton et al., 2015; Kish et al., 2015; Vlachopoulos et al., 2015). Literature review reveals that countermovement jumping exercise could increase bone strength (Babatunde & Forsyth, 2014; Montgomery et al., 2020b; Niu et al., 2010; Vlachopoulos et al., 2018a).

Countermovement jumping exercise is recommended for enhancing bone strength and muscular performance as it involves combination movement of extensor and flexor muscle of the legs which led by a high concentric activation to obtain moderate eccentric activation, thus demands a gradual activation of motor units (Sánchez-Sixto et al., 2018; Śliwowski et al., 2018). This resulting in improvement of muscle strength and power. According to Tavafzadeh et al. (2011), during an impact exercise, muscle contraction acts on bone shaft surface, stimulates bone adaptation and increases bone mass. Therefore, bone strength is developed and subsequently improve bone health status. According to Robling et al. (2002), six hours rest between jumping exercise sessions is important to stimulate osteogenic effects on bone (Robling et al., 2002).

Jumping exercise is a type of simple exercise with 3-4 minutes for 1 session of jumping exercise generally. The height of jumping can be based on jumping height determination using force platform and Quatro software. Jumping exercise could improve muscular power and strength by generating lower limb muscle force in every jumping action.

Other than inactive lifestyle, the major factors that can cause low BMD are poor body anthropometry (Chin et al., 2012b), disturbance of reproductive hormones (Chin & Ima-Nirwana, 2012c), sarcopenia (Verschueren et al., 2013), unhealthy lifestyle (alcoholism) (Berg et al., 2008) and diseases such as metabolic syndrome and diabetes (Yaturu et al., 2008). Bone resorption and bone formation are tightly coupled in bone remodelling process which involves osteoblast and osteoclast. The imbalance between osteoblast and osteoclast activity could be influenced by oxidative stress which leads to bone loss (Schramm et al., 2003).

Regarding bone turnover markers, carboxyterminal telopeptide of type-1 collagen (1CTP) (bone resorption marker) and bone-specific alkaline phosphatase (BALP) (bone formation marker) are commonly used to reflect bone turnover or bone metabolism (Ooi & Sahrir, 2018; Zuhri et al., 2020). Bone turnover markers have also been used to assess the risk of osteoporosis (Szulc & Delmas, 2008) and monitoring osteoporosis (Vasikaran, 2008). Commonly, methods that have been used for the measurements of bone turnover markers including enzyme-linked immunosorbent assays (ELISA), radioimmunoassay, immunoradiometric assay and chemiluminescence assay (Burch et al., 2014; Shetty et al., 2016).

Oxidative stress refers to the production of high level of reactive oxygen species (ROS) (e.g., superoxide and hydrogen peroxide) that cause damage to lipids, protein and DNA (Pisoschi & Pop, 2015; Schieber & Chandel, 2014). According to Wauquier et al. (2009), ROS can encourage the osteoclast resorption directly by simulating RANK signalling and stimulating osteoclast differentiation or indirectly by

stimulating both osteoblast and osteoclast coupling and followed by osteoclast differentiation supported by RANKL expression. Therefore, high oxidative stress level will cause high risk of bone loss, and an individual will be prone to have osteoporosis if these conditions is not controlled.

Protein oxidation is one of the indirect biomarkers of exercise-induced oxidative stress (Bloomer et al., 2007; Wadley et al., 2016a). Commonly, protein carbonyl concentration is determined in analysis of biomarkers of protein oxidation. Change in protein carbonyl concentration can be influenced by the exercise duration and exercise intensity (Wadley et al., 2016b).

In men, testosterone is important for adaptations to resistance exercise and training (Vingren et al., 2010). Testosterone is required for the anabolic stimulation process and constrains degradation of protein (anti-catabolic effect) that is crucial for promotion of skeletal muscle growth in response to exercise (Demling & Orgill, 2000; Thomas et al., 2009b). Generally, in men, circulating total testosterone and free testosterone upsurge immediately after a session of heavy resistance training and return to or below the baseline within 30 minutes (Kraemer et al., 2006; Kvorning et al., 2007; Orwoll et al., 2012). The change in testosterone level is greatly influenced by the exercise intensity, rest duration, number of sets, and type of exercise prescribed (Vingren et al., 2010).

There are various types of supplement that can help to promote bone health such as calcium (Nakamura et al., 2012; Rajatanavin et al., 2013; Reid et al., 2008), milk (Gui et al., 2012; Manios et al., 2007), Vitamin D (Grimnes et al., 2012; Steffensen et al., 2011; Verschueren et al., 2011) and dairy milk (Gui et al., 2012; Manios et al., 2007). Previous studies revealed that these supplementations could enhance bone mineral density, trigger bone formation, and reduce the risks of getting osteoporosis.

Channa Striata (CS) or snakehead fish is a freshwater, air-breather and carnivorous fish indigenous to many tropical countries (Baie & Sheikh, 2000a). *Channa Striatus* is known locally to the Malay as Haruan and it is widely consumed in Malaysia and other Southeast Asian countries (Zakaria et al., 2005a). There are 30 species of snakehead fish in that family reported around the world and eight of them are found in Malaysia. This snakehead fish belongs to Channidae family. The members of the Channidae family are also found in Myanmar, Thailand, Laos, Cambodia, Vietnam, Brunei, Philippines, Indonesia and Singapore (Ali Khan et al., 2014). *Channa Striatus* is a wild species and a top predator in shallow, slow-moving waters with temperatures between 20-30^{o}C, while it is also a good survivor in tough environment with low dissolved oxygen and low turbidity (Shafri & Abdul Manan, 2012). **Figure 1.1** shows the photo of snakehead fish (*Channa Striata*).

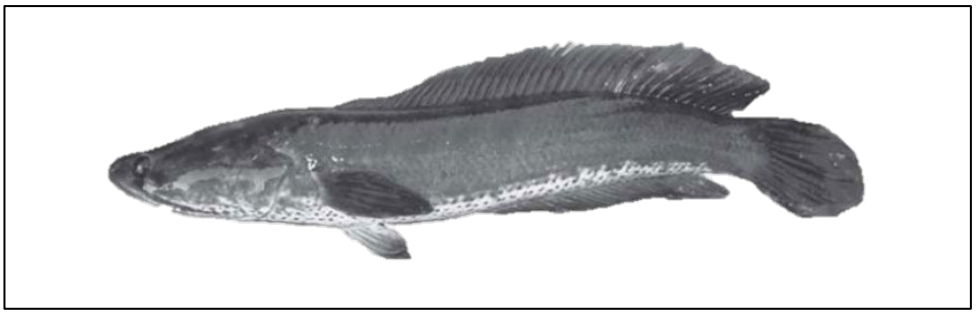

Figure 1.1 The snakehead fish (*Channa Striatus*)

Adapted from Shafri and Abdul Manan (2012)

According to Courtenay and Williams (2018), the name "snakeheads" was given since species in the family Channidae have large sizes of wide heads relatively when compared to that of snakes. Moreover, snakehead fish have black-brown striped on the upper section of its body and white bands on its belly. The size of the striped of female snakehead fish is larger than the male. They are also having sharp teeth with 4 to 7 canines located on the bottom of their large mouth.

Channa Striatus contains biochemical components and albumin which can elicit beneficial effects to the consumers. *Channa Striatus* appears to have a medium level of antioxidant activities possibly contributed by albumin contained in *Channa Striatus* which act as radical scavenging properties (Dahlan-Daud et al., 2010; Suhartono et al., 2013). Albumin contains many sulfhydryl groups (-SH) that can serve as radical binder and plays an important role to inhibit free radical production by polymorphonuclear leukocytes. The antioxidants present in *Channa Striatus* are lipophilic antioxidants which represent powerful defence tools particularly against

oxidative stress, malondialdehyde (MDA) and reactive oxygen species (ROS) (Suhartono et al., 2013). Thus, exogenous antioxidant supplements such as *Channa Striatus* can be a suitable non-invasive tool in reducing oxidative stress.

A few previous studies showed that *Channa Striatus* contains biochemical components such as essential amino acids and fatty acids (Baie & Sheikh, 2000b; Laila et al., 2011; Sahid et al., 2018) and also glycine, a non-essential amino acid (Zakaria et al., 2007) in *Channa Striatus* extract. Moreover Dahlan-Daud et al. (2010) mentioned that there are other non-essential amino acid are discovered, such as glutamic acids, arginine, glycine, proline, alanine and aspartic acid, meanwhile essential amino acids are found such as leucine and lysine. *Channa Striatus* has high contents of arachidonic acid and polyunsaturated fatty acids that can accelerate prostaglandin synthesis (Jais & Manan, 2007; Shafri & Abdul Manan, 2012) which plays a crucial role in healing the wounds. Zakaria et al. (2005a) mentioned that the entire fish can be a dietary medicine which can be consumed by grilling, dry-frying, or boiling in porridge for wound healing. General content of *Channa Striatus* is shown in Table 1.1.

Table 1.1 General content of *Channa Striatus*

Amino acids (g/100g) (Gam et al., 2005)	
Glutamic acid	13.42-14.57
Aspartic acid	8.36-9.37
Lysine	8.61-9.13
Arginine	8.39-9.18
Leucine	8.35-8.87
Alanine	5.78-6.04
Valine	5.14-4.67
Threonine	5.15-5.50
Serine	4.70-5.23
Phenylalanine	4.64-5.09
Glycine	4.55-5.97
Isoleucine	4.49-5.23
Tyrosine	4.02-4.31
Proline	3.58-4.08
Methionine	3.14-3.92
Histidine	2.49-3.06
Cysteine	0.88-1.64
Fatty Acid (%) (Zakaria et al., 2007)	
Palmitic acid (C16:0)	35.93 ± 0.63
Oleic acid (C18:1)	22.96 ± 0.40
Stearic acid (C18:0)	15.31 ± 0.33
Linoleic acid (C18:2)	11.45 ± 0.31
Arachidonic acid (C20:4)	7.44 ± 0.83
Heptadecanoic acid (C17:0)	2.90 ± 0.56
Myristic acid (C14:0)	2.15 ± 0.11
Palmitoleic acid (C16:1)	1.86 ± 0.32
Mineral contents (mg/kg) (Paul et al., 2013)	
Calcium (Ca)	5279
Magnesium (Mg)	276
Zinc (Zn)	8.30
Iron (Fe)	4.93
Lead (Pb)	0.06
Mercury (Hg)	0.01
Chromium (Cr)	0.01
Nutrient (g/100g) (Mustafa et al., 2012)	
Protein	3.36 ± 0.29
Albumin	2.17 ± 0.14
Fat	0.77 ± 0.66
Glucose	0.07 ± 0.02

Chantiva 750 Tablet manufactured by Major Interest Sdn. Bhd. had been used in this study as *Channa Striatus* supplementation with registration number (MAL16070006TC) and HALAL was certified with reference number JAKIM/(S)/ (22.00/492/2/1038-07/2013). Table 1.2 shows the active ingredients of Chantiva 750 Tablet.

Table 1.2 General content of Chantiva 750 tablet

Ingredients	**Content (mg)**
Semen Nigella Sativa	25
Channa Striatus (Whole fish)	500

To date, information on combined effects of *Channa Striatus* and countermovement jumping exercise on bone parameters, muscular performance and antioxidant status in young inactive males is still lacking, therefore the present study was proposed.

1.2 Problem Statements

Numerous studies have been done on the use of *Channa Striatus* for wound healing, osteoarthritis, anti-inflammatory activities, and as source of albumin. Studies of this fish extract showed that it was a good source of both amino acids and fatty acids, and it is postulated to have impact on muscular and bone health. However, there is paucity of study on the effect of *Channa Striatus* on muscular performance and bone health. Eight weeks of jumping exercise has been reported could significantly efficient

to improve bone mineral density in animals (Honda et al., 2001; Ooi et al., 2009). However, to date information on the length of the jumping exercise intervention period for eliciting bone health in human is still limited. Thus, 12 weeks of jumping exercise was proposed as the intervention period for the present study. This study aims to evaluate the effects of 12 weeks combined *Channa Striatus* supplementation and countermovement jumping exercise on bone parameters, muscular performances, and antioxidant status in young physically inactive males.

1.3 Objectives of the Study

General objective:

To investigate the combined effects of *Channa Striatus* supplementation with countermovement jumping exercise for a duration of 12 weeks on bone parameters, muscular performance, and antioxidant status in young physically inactive males.

Specific objectives:

a. To determine the differences of bone parameters among sedentary without *Channa Striatus* supplement control group (C), *Channa Striatus* supplement group (CS), countermovement jumping exercise group (J) and combined *Channa Striatus* and countermovement jumping exercise group (CSJ).

b. To determine the differences of muscular performance among sedentary without *Channa Striatus* supplement control group (C), *Channa Striatus* supplement

group (CS), countermovement jumping exercise group (J) and combined *Channa Striatus* and countermovement jumping exercise group (CSJ).

c. To determine the differences of antioxidant status among sedentary without *Channa Striatus* supplement control group (C), *Channa Striatus* supplement group (CS), countermovement jumping exercise group (J) and combined *Channa Striatus* and countermovement jumping exercise group (CSJ).

1.4 Hypotheses

H_{01}: There are no significant differences on bone parameters in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa Striatus* supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

H_{A1}: There are significant differences of bone parameters in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa Striatus* supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

H_{02}: There are no significant differences of muscular performance in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa Striatus* supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

H_{A2}: There are significant differences of muscular performance in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa*

Striatus supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

H_{03}: There are no significant differences of antioxidant status in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa Striatus* supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

H_{A3}: There are significant differences of antioxidant status in combined *Channa Striatus* supplementation and jumping exercise group compared to *Channa Striatus* supplementation group, jumping exercise group and sedentary without *Channa Striatus* consumption control group.

1.5 Significance of the Study

To date, there is limited published data regarding the effects of *Channa Striatus* on bone parameters, muscular performance, and antioxidant status. Previous study used *Channa Striatus* for wound healing in rats, reducing pain after post-caesarean in women and test as anti-ulceration in gastrointestinal in rats. This project is worth to be carried out to provide information on the influence of combined *Channa Striatus* supplementation and jumping exercise on other aspect of parameters. Other than that, this combination can be proposed as guideline in planning exercise and nutritional programs in young males.

Channa Striatus contains fatty acid (arachidonic acid), amino acid (glycine), and m,antioxidant (albumin) which are important to maintain bone health (Muhamad

& Mohamad, 2012; Zuraini et al., 2006) and reduce oxidative stress (Dahlan-Daud et al., 2010; Suhartono et al., 2013) in human. *Channa Striatus* supplementation used in the present study contains 500mg of Channa Striatus in one tablet and is ready to be consumed. Participants were required to consume only one tablet per day in the morning. In terms of exercise, the prescribed jumping exercise programme was based on a previous study by Erickson and Vukovich (2010). The number of jumping is progressively increased to keep up with individual physiological changes and adaptation in human body.

It is hoped that the present study findings provide community the information of the effective exercise that is enjoyable, low cost and helpful in improving the bone mineral density and muscular strength during young age and reduce the risk of getting osteoporosis later in life. The findings will contribute to sport nutrition development as well.

1.6 Operational Definition

***Channa Striatus* supplementation:** The supplement is commercially available and was supplied by Major Interest Sdn. Bhd. This supplement is in tablet form and consumed by participants in *Channa Striatus* (CS) group and combined *Channa Striatus* and jumping (CSJ) group. The participants consumed one tablet (750mg) per day in the morning for 12 weeks.

Countermovement jumping exercise: The countermovement jump (CMJ) exercise involves combination complex movement of extensor and flexor muscle of the legs

which initiate by a high concentric activation to obtain moderate eccentric activation, thus demands a gradual activation of motor units.

Muscular Performance: It reflects capacity of a group of muscles to generate force producing movement or maintaining the position of parts of the body that are required for functional activity. Muscle strength is the muscle force exerted by specific muscle to overcome resistance under a specific circumstance. Muscle power is the ability to exert out a maximal contraction on a short period of time.

Antioxidant Status: An antioxidant is a molecule that is capable to inhibit the oxidation of another compound. Human serum albumin is one of the antioxidant components that is soluble, globular, and unglycosylated monomeric protein which produced in human hepatocytes. Albumin plays a vital role in antioxidant defence under both normal and oxidative stress conditions.

Oxidative stress: Oxidative stress is the imbalance between production of free radicals and an antioxidant in cells and tissues. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid peroxidation in cells. Increase amount of MDA in serum is a sign of oxidative stress.

Protein Carbonyl: Protein carbonylation is the type of protein oxidation promoted by reactive oxygen species. Protein carbonylation is possibly resulted from direct

oxidation of lysine, arginine, proline, and threonine residues and interaction with reactive carbonyl species produced from carbohydrate and lipid oxidation.

Testosterone: Testosterone is crucial for enhancing muscle mass, fat loss and bone health. Impact exercise can boost the testosterone concentration in the body. Decreased level of testosterone can lead to osteoporosis and weak muscle.

Bone-specific alkaline phosphatase (BALP): BALP is a biochemical marker of bone formation and the activity of ALP is important for the mineralization of the bone. High in serum ALP concentration reflects high in osteoblast activity on the bone surface.

Carboxyterminal telopeptide of type 1 collagen (1CTP): 1CTP is a bone resorption marker. 1CTP reflects the breakdown of type 1 collagen largely derived from bone. Increased level of serum 1CTP showed increased in osteoclast activity resulting the bone matrix degradation.

Speed of sound (SOS): Bone SOS is an indicator of bone health status, which can reflect bone mineral density. Bone SOS was measured by using quantitative ultrasound bone sonometer in the study. Ultrasonic sound wave travel through the bone and wave changes by the structure and content of the bone. The more complex structure of the bone produces high reading of speed of sound.

CHAPTER 2

LITERATURE REVIEW

2.1 A Conceptual Framework for Bone Health and Muscular Performance

Previous cross-sectional studies reported that there was positive relationship between bone and muscle properties in youth population (Janz & Baptista, 2018; Janz et al., 2015; Macdonald et al., 2006). However, there was non-modifiable factors such as age (Mishra et al., 2016), gender, diseases (Vijayakumar & Büsselberg, 2016), and genetics (Chastin et al., 2014) and modifiable factors such as nutrition intake (Fratoni & Brandi, 2015), smoking and alcohol intake (Abrahamsen et al., 2014), physical activity (Gracia-Marco et al., 2011; Santos et al., 2017) which contributed to the bone health and muscle.

Figure 2.1 demonstrated a conceptual framework for the modifiable and non-modifiable factors that contributes to bone health and muscular performance. According to Daly et al. (2014), regular exercise and adequate nutrition intake such as dietary calcium, vitamin D and protein, were prescribed as strategies to enhance peak bone mass and maintain both bones and muscles health.

Regarding bones, exercise is required to stimulate bone modelling and remodelling, while calcium, vitamin D, and other nutrients are necessary substrates for bone mineralization (Specker & Vukovich, 2007). Meanwhile, for muscles, exercise and adequate nutrition were beneficial to improve the balance between muscle protein

synthesis and breakdown, resulting in a net positive protein balance to induce muscle hypertrophy (Koopman et al., 2007).

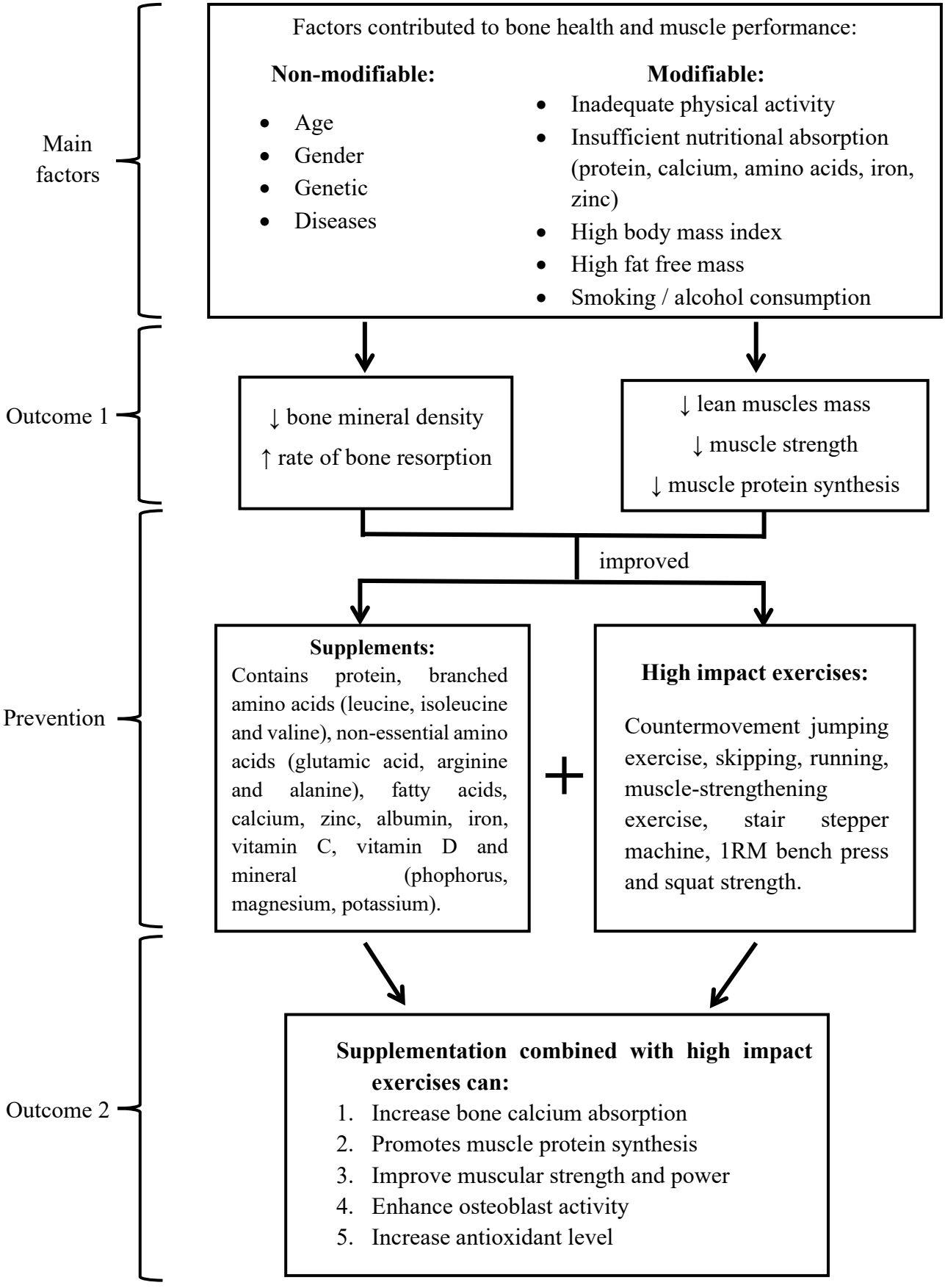

Figure 2.1 A conceptual framework for the factors that contributes to bone health and muscular performance

2.2 *Channa Striatus* Supplement

2.2.1 Nutritional content and benefits of *Channa Striatus*

Channa Striatus or Haruan is a fresh-water snakehead fish and has been commonly used traditionally for centuries as a remedy to treat the wound and reduce post-operation pain in Malaysia. Zakaria et al. (2005a) mentioned that the entire fish could be a dietary medicine consumed by grilling, dry-frying, or boiling in porridge for wound healing. Among all types of *Channa* species, *Channa Striatus* is the type that is the most recommended species to be used as a supplement or remedy to reduce pain and chronic inflammation (Zuraini et al., 2006). *Channa Striatus* contains fatty acid, albumin, and amino acid (Baie & Sheikh, 2000a). Those major bioactive compounds were believed could help to treat inflammation and dysfunction of tissues and organs.

Epidemiological evidence shows that various fatty acid categories include n-9 monounsaturated fatty acids (MUFA), n-3 and, n-6 polyunsaturated fatty acids (PUFA), which are beneficial to health (Bjermo et al., 2012; Senanayake et al., 2014). There are two essential fatty acids, which are α-linoleic acid (ALA), the parent of the n-3 fatty acid family, while linoleic acid (LA), the parent of the n-6 fatty acid family (Saunders et al., 2013). The essential fatty acid cannot be synthesized by the human body, so it must be provided through the diet. Linoleic acid and α-linoleic acid can be converted into long-chain PUFAs by enzymes (Calder, 2012). Linoleic acid is a precursor of arachidonic acid (AA), and ALA is a precursor of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) (Saunders et al., 2013). According to Lau et al. (2013) and Gammone et al. (2019), n-3

polyunsaturated fatty acids (PUFA) can affect skeletal muscle metabolism and beneficial to maintaining bone health and even bone strength. There are EPA, DHA, and AA content in *Channa Striatus* extract (Muhamad & Mohamad, 2012; Zuraini et al., 2006) that are beneficial to the bone.

A previous animal study done on male Sprague-Dawley rats by Lau et al. (2010) found that rats that consumed n-3 and n-6 PUFA for nine weeks had statistically significant higher values of stiffness and peak load compared to the control group after adjustment of body weight which indicated that the rats have strong bone. In addition, Lukas et al. (2011) reported that female rats Sprague-Dawley rats that consumed tuna oils for eight weeks, rich in DHA, had higher tibial bone mineral density and bone mineral content.

Evidence indicates that chances of getting hip fracture were reduced to 80% in men with a high intake of arachidonic acid (Farina et al., 2011a). Arachidonic acid is believed can affect the receptor activator of nuclear factor κβ (RANK), a receptor found on the osteoclast, the cell causing bone resorption, which controls osteoclast formation (Kruger et al., 2010). Additionally, food that contains high fatty acid content is also believed can promote cells to stimulate prostaglandin synthesis and subsequently work as a mediator if any inflammation occurs in tissues and organs (Baie & Sheikh, 2000b).

Besides fatty acids, amino acids are also the major compound in *Channa Striatus*. The examples of amino acids are glutamic acid, glycine, leucine, aspartic acid,

proline, alanine, and arginine with values 1.87-43.13 mg/g, 21.80-80.85 mg/g, 7.85-40.19 mg/g, 13.85-44.07 mg/g, 9.49-45.46 mg/g, 11.38-35.25 mg/g and 5.99-21.79 mg/g, respectively (Dahlan-Daud et al., 2010). The branched-chain amino acids (BCAA) such as leucine, isoleucine, and valine are three of nine essential amino acids, and the other eleven non-essential amino acids are also important for new muscle protein synthesis (Estoche et al., 2019). BCAAs account for 35–40% of the dietary essential amino acids in body protein and 14–18% of the overall amino acids in muscle proteins (Shimomura et al., 2006). Recent studies showed that free BCAAs, especially leucine, play a very important role in protein metabolism, especially leucine, which promotes muscle protein synthesis and inhibits protein breakdown (Beaudart et al., 2018; Estoche et al., 2019). These findings suggest that leucine is not only a building block of proteins but also a modulator of protein metabolism (Wu, 2013; Zhang et al., 2017).

Cao and Nielsen (2010) mentioned that the intake of amino acids could maintain bone mineral density by increasing intestinal calcium absorption. A study on postmenopausal women showed that the intake of amino acid significantly reduces hip fracture incidence, increase muscular strength and reduce the risk of medical complication (Bonjour, 2011). A previous study done on trained males by Howatson et al. (2012) showed that BCAAs supplement group showed a significantly decreased muscle soreness and greater recovery of maximal voluntary contraction compared to placebo group. From this background, it is interesting to consider the efficacy of these amino acids are ingested as a supplement and the nutraceutical effect of these amino acids on skeletal muscle in relation to exercise.

Among freshwater fishes, *Channa Striatus* appears to have a medium level of antioxidant activities, which be contributed by albumin contained in *Channa Striatus.* Albumin can act as radical scavenging properties (Dahlan-Daud et al., 2010; Suhartono et al., 2013) because it contains many sulfhydryl groups (-SH), which serve as a radical binder and plays an important role to inhibit free radical production by polymorph nuclear leukocytes. The antioxidants present in *Channa Striatus* are most likely to be lipophilic antioxidants which represent powerful defense tools, particularly against oxidative stress such as malondialdehyde (MDA) and reactive oxygen species (ROS) (Suhartono et al., 2013). Therefore, exogenous antioxidant supplements like *Channa Striatus* can be a suitable non-invasive tool in reducing oxidative stress.

Based on the literature search, most of the previous studies of *Channa Striatus* were carried out in animals such as rats (Jais et al., 1997; Suhartono et al., 2013; Zakaria et al., 2007) and rabbits (Michelle et al., 2004) while in humans were done on patients with knee osteoarthritis (Azidah et al., 2017) and post lower segment caesarean section (Bakar et al., 2015; Shafii et al., 2017; Wahab et al., 2015) was reported. In the market, *Channa Striatus* has been formulated to be packaged as an extract (Shafri & Abdul Manan, 2012), fillet (Jais et al., 1994), cream (Baie & Sheikh, 2000a), and spray (Laila et al., 2011) and was tested in animal studies.

Moreover, *Channa Striatus* was reported to have the potential to protect against oxidative stress (Suhartono et al., 2013), elicit antinociceptive effects to reduce pain (Zakaria et al., 2007), beneficial for wound healing (Baie & Sheikh, 2000a), elicit

protective effect against toxic (i.e., cadmium) (Suhartono et al., 2013), increase the tensile strength of skin and remodel the collagen (Baie & Sheikh, 2000a), work as an energy booster (Shafri & Abdul Manan, 2012), initiate blood clotting, responsible for growth (Jais et al., 1994), reducing pain (Wahab et al., 2015) and inflammation (Zuraini et al., 2006).

2.2.2 Effects of *Channa Striatus* on osteoarthritic treatment

Channa Striatus supplementation had been consumed by patients with osteoarthritis. Based on the previous findings, there were improved bone health in osteoarthritis patients after consuming *Channa Striatus* supplement. Consumption of *Channa Striatus* supplement could improve knee osteoarthritis symptoms such as pain based on Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaires after three months of the intervention period (Kadir et al., 2014). Meanwhile, Western Ontario and McMaster University Osteoarthritis Index (WOMAC) stiffness and function scores (Azidah et al., 2017) were also reduced after six months of consumption of a similar supplement. Azidah (2018) added that *Channa Striatus* also had an effect as an anti-inflammatory, wound healing, antioxidant, and analgesic properties.

Meanwhile, in animal studies, Azidah et al. (2019) reported that osteoarthritis rabbit showed an increase in thickness, area, and roughness of cartilage after consumed 51.4 mg/kg of *Channa Striatus* extract for eight weeks and speculated that *Channa Striatus* extract elicits chondroprotective action in maintaining the structure of the cartilage. However, a low dosage of *Channa Striatus* extract, 15 ml/kg can increases

the PGP 9.5 immunoreactive nerve fibres in the synovial membrane in rats due to the active components in the supplement that can help in increasing the nerve fibres and reduced the inflammatory cells (Ganabadi, 2009). These findings also in line with a study done by Michelle et al. (2004) on rabbits with osteoarthritis, which consumed 10 ml/kg of *Channa Striatus* extracts for nine weeks.

2.2.3 Effects of *Channa Striatus* on wound healing

Wound healing is a complex process involving several overlapping stages that include inflammation, granulation tissue formation, re-epithelialization, matrix formation, and scar contraction with remodelling (Kumar et al., 2006; Laila et al., 2011; Rahman et al., 2018). The collagen determines strength and structure in normal tissues. Therefore, collagen is one of the essential components contributing to enhancing the tensile strength of the wounded skin (Laila et al., 2011). According to Steed (2003), adequate nutrition intake is required to enhance fibroblast cell proliferation to produce collagen fibre network in wound healing. Overall, consumption of *Channa Striatus* supplement has the potential to be an exogenous supplement that could give beneficial effects on wound healing processes.

A study done on Post Lower Segment Caesarean Section Women (LSCS) by Bakar et al. (2015) found that there was improvement in longitudinal and oblique transverse plane of uterine anterior posterior measurement of the uterus after six weeks of 500 mg *Channa Striatus* extract consumption. The authors also found positive findings on Visual Analogue Scale Scores (VAS) and Patient Satisfaction Score (PSS). A previous study by Sahid et al. (2018) showed that there was a significant