THE EFFECTS OF PROPHYLACTIC AND THERAPEUTIC INTERVENTIONS OF NIGELLA SATIVA ON FUNCTIONAL AND BIOCHEMICAL MARKERS OF MUSCLE DAMAGE AND INFLAMMATION FOLLOWING ECCENTRIC EXERCISE

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LIST OF ABBREVIATION

EIMD	Exercise-induced muscle damage
DOMS	Delayed onset muscle soreness
COX	Cyclooxygenase
NSAIDs	Nonsteroidal anti-inflammatory drugs
TQ	Thymoquinone
NS	Nigella sativa
VAS	Visual analog scale
ROM	Range of motion
CK	Creatine kinase
LDH	Lactate dehydrogenase
ROS	Reactive oxygen species
IL-1β	Interleukin-1β
IL-6	Interleukin-6
MVC	Maximal voluntary contraction
FANG	Flexed angle
RANG	Relaxed angle
DHR	Downhill running
CMJ	Counter-movement jump

KESAN INTERVENSI NIGELLA SATIVA SECARA PROFILAKTIK DAN TERAPUTIK KE ATAS PENANDA-PENANDA KEFUNGSIAN DAN BIOKIMIA KECEDERAAN OTOT DAN INFLAMASI SELEPAS LATIHAN ESENTRIK

ABSTRAK

Nigella sativa (NS) telah diketahui dengan meluas mempunyai kesan-kesan anti-inflamasi ke atas pelbagai model inflamasi, namun belum dijumpai kajian yang dilakukan ke atas kecederaan otot yang diakibatkan oleh latihan (EIMD) sebagai model inflamasi. Kajian pertama yang telah dilakukan oleh pasukan penyelidik yang sama gagal untuk menentukan dos yang efektif untuk meningkatkan pemulihan selepas EIMD kerana cara pengambilan NS hanya satu kali sahaja tidak memberi sebarang kesan ke atas semua parameter yang diukur. Maka, dalam kajian ini, cara pengambilan NS telah ditukarkan daripada satu kali sahaja kepada beberapa kali secara berterusan untuk melihat keberkesanan NS dalam mencegah dan merawat EIMD. Objektif kajian ini adalah untuk menentukan keberkesanan intervensi NS secara profilaktik dan teraputik dalam membantu pemulihan selepas latihan. Tiga puluh orang lelaki sedentari berusia 19 hingga 27 tahun telah dibahagikan secara *double-blind* kepada 3 kumpulan, sama ada profilaktik (P), teraputik (T) atau kawalan (C). Kumpulan P mengambil NS 2000mg/hari bermula 2 minggu sebelum latihan sehingga 4 hari selepas latihan, manakala kumpulan T mengambil NS 2000mg/hari bermula 30 minit sebelum latihan sehingga 4 hari selepas latihan. Kumpulan C pula mengambil placebo. Mereka menjalani *counter-movement jump (CMJ)* sebanyak 10 set X 10 ulangan dengan 30 saat rehat antara set, dan berada

dalam keadaan *squat* sewaktu permulaan dan pendaratan untuk mencetuskan kecederaan kepada otot. Penanda-penanda kefungsian (kuasa otot, kesakitan, ketegangan, ukur lilit peha, *range of motion* (ROM)) dan penanda-penanda biokimia (enzim darah iaitu *creatine kinase* (CK) dan *lactate dehydrogenase* (LDH)), *cytokine* (interleukine-6 (IL-6) dan interleukine-1β (IL-1β)), serta sel darah putih (neutrofil dan makrofaj)) bagi kecederaan otot telah diukur sebelum latihan, 30 minit, 1 , 2, 3 dan 4 hari selepas latihan. Analisis menggunakan *mixed-factorial* ANOVA menunjukkan tiada interaksi yang signifikan ditemukan ke atas kuasa otot, kesakitan, ketegangan, ukur lilit peha, *range of motion* (ROM), *creatine kinase* (CK), interleukine-6 (IL-6), interleukine-1β (IL-1β) dan makrofaj di antara ketiga-tiga kumpulan yang berbeza pada setiap sesi pengukuran. Ini bermakna tiada perbezaan yang signifikan dijumpai terhadap penanda-penanda tersebut tidak kira intervensi mana yang diberi kepada kepada peserta. Walau bagaimanapun, terdapat interaksi yang signifikan dijumpai pada penanda *lactate dehydrogenase* (LDH) dan bilangan neutrofil di antara ketiga-tiga kumpulan pada setiap sesi pengukuran. *Lactate dehydrogenase* (LDH) dan bilangan neutrofil tidak begitu terkesan dalam kumpulan P dari pengukuran sebelum latihan sehingga 4 hari selepas latihan jika dibandingkan dengan kumpulan T dan C yang mengalami penurunan ketara dan peningkatan mendadak bagi *lactate dehydrogenase* (LDH) dan bilangan neutrophil. Pengambilan NS pada 2 minggu sebelum dan berterusan sehingga pemulihan selepas latihan telah memberi manfaat untuk menurunkan magnitud peningkatan *lactate dehydrogenase* (LDH) dan menurunkan *lactate dehydrogenase* (LDH) kepada nilai sebelum latihan dengan lebih cepat, berbanding pengambilan NS pada hari latihan sehingga pemulihan. Ini menerangkan bahawa NS dapat mempercepatkan proses pemulihan selepas EIMD jika diambil 2 minggu sebelum latihan sehingga pemulihan.

Kesimpulannya, pengambilan NS dua minggu sebelum dan berterusan sehingga pemulihan (secara profilaktik) selepas latihan memberi manfaat dalam mengurangkan magnitud peningkatan *lactate dehydrogenase* (LDH) dan neutrofil setelah EIMD dan mempercepatkan penurunan penanda-penanda kecederaan kepada nilai sebelum latihan, berbanding pengambilannya pada hari latihan berterusan sehingga pemulihan (secara teraputik), dan ini membuktikan bahawa NS berpotensi digunakan sebagai *suplemen* pencegahan, bukan rawatan selepas kecederaan.

THE EFFECTS OF PROPHYLACTIC AND THERAPEUTIC INTERVENTIONS OF NIGELLA SATIVA ON FUNCTIONAL AND BIOCHEMICAL MARKERS OF MUSCLE DAMAGE AND INFLAMMATION FOLLOWING ECCENTRIC EXERCISE

ABSTRACT

Nigella sativa (NS) is widely known to have anti-inflammatory effects on various inflammation models, however no known study has been done on exercise-induced muscle damage (EIMD) as a model of inflammation. The first study that has been carried out by the same researchers before was unable to determine the effective dose to enhance recovery following EIMD because the single one-off dose method used in the study failed to affect all measured parameters. Thus, in this study, the method of supplementation was changed from a single one-off dose to prolonged multiple-doses to see the effectiveness of NS on prevention and treatment of EIMD. The objective of this study was to determine the effectiveness of prophylactic and therapeutic interventions of NS in enhancing recovery following exercise. Thirty sedentary men aged 19 to 27 y.o were double-blindedly assigned to 3 groups, either prophylactic (P), therapeutic (T) or control (C). P group administered 2000mg/day of NS daily from 2 weeks before exercise to four days post-exercise, meanwhile T group administered 2000mg/day of NS from 30 min before exercise bout to four days post-exercise. The third group (C group) was a control group, which was administered placebo. They had undergone 10 sets X 10 repetitions of counter-movement jump (CMJ) with 30 second rest between each set, employing squat in starting and landing position to facilitate muscle damage. A set of tests were conducted to measure functional markers (muscular power, soreness, tenderness, thigh circumference, range of motion (ROM)) and biochemical markers (blood enzymes (i.e. creatine kinase (CK) and lactate dehydrogenase (LDH)), cytokines (interleukin-6 (IL-6) and interleukin-1β (IL-1β)), and white blood cells (neutrophils and macrophages)) of muscle damage were measured at pre-exercise, 30 minutes, 1, 2, 3 and 4 days post-exercise. Analysis by a mixed-factorial ANOVA showed that no significant interactions were found on muscle power, soreness, tenderness, thigh circumference, range of motion (ROM), creatine kinase (CK), interleukin-6 (IL-6), interleukin-1β (IL-1β) and macrophages between three different groups across the measurement sessions. It means there were no significant differences regardless of which intervention the participants had been given on these parameters. However, there were significant interactions found on lactate dehydrogenase (LDH) and neutrophils between three different groups across the measurement sessions. Lactate dehydrogenase (LDH) and neutrophils count were not so much affected in P group from pre-exercise to post-4 days measurement compared to T and C group, which experienced abrupt decrease and sudden increase in their lactate dehydrogenase (LDH) and neutrophils count. Ingesting NS two weeks prior and continuously until recovery following exercise was found to have benefit in lowering the magnitude of increase in lactate dehydrogenase (LDH) and returning lactate dehydrogenase (LDH) to pre-exercise count faster, compared to ingesting NS on the exercise day until recovery. This explained that NS can speed the recovery process following EIMD if taken two weeks before engaging in exercise until recovery. In conclusion, ingesting NS two weeks prior and continuously until recovery (prophylactically) following exercise was found to have benefit in lowering the magnitude of increase in lactate dehydrogenase (LDH) and neutrophils after EIMD and returning the markers to pre-

exercise count faster, compared to ingesting NS on the exercise day until recovery (therapeutically), proving that NS can be used as a potential preventive supplement, not as an after-injury treatment.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

'Pain' is a very common word in sports. It can be a reason that refrain people from sports. The sensations of pain, muscle tightness, swelling and tenderness that may arise following an exercise, especially when the exercise is unaccustomed, intense or prolonged are widely recognized as delayed-onset muscle soreness (DOMS) (Close *et al.*, 2005).

DOMS has been reported by Howatson and van Someren (2008) to be the evidence of exercise-induced muscle damage (EIMD) besides losses of strength and power, local swelling, stiffness, structural disruption and increase in proteins activities within the muscles and in the circulation. EIMD is also a result of morphological changes within the muscles reduces agility and reduces proprioception. It may appear from one to three days following exercise and clear within a week following exercise. (Hortobagyi *et al.*, 1998; Lauritzen *et al.*, 2009; Highton *et al*., 2009; Saxton *et al.*, 1995).

The occurrence of EIMD is not permanent. It is a repairable damage that caused by eccentric contractions. Compared to concentric contractions, eccentric contractions cause greater and remarkable damage to the muscle tissues. This is because the muscles are lengthened by force in an eccentric exercise. As we know, the nature of the muscles is to contract voluntarily, not to forcibly lengthen. Windju (2011) reported that muscles are hardly lengthening by force compared to contracting by force. According to Proske and Morgan (2001), eccentric exercise involves braking system, in which the muscles are eccentrically contract to slow or stop the motions that are caused by concentric contractions. This system requires high-tension forces that can cause muscles damage easier.

The damage to the muscles initiates a series of event that lead to the repair and restoration of the muscle function. This series of event are called inflammatory response (Malm, 2001). It starts when the neutrophils are activated into the circulation by the damaged muscle. After several hours, they signal macrophages to infiltrate the damaged tissue and it remains present in the circulation up to several days after exercise (Malm *et al.*, 2000). Their functions during the initial inflammatory response are to produce pro-inflammatory cytokines, and prostaglandins. Prostaglandins are produced by cyclooxygenase (COX) enzyme, and its functions are to enhance and to reduce inflammation. Due to the accumulation of prostaglandins, blood vessels become more permeable and this can increase swelling at the involved muscles group. It can also sensitize mechanoreceptors and nociceptors that lead to the feeling of pain, as well as improve the protein deterioration process (Ricciotti and FitzGerald, 2011; Windju, 2011).

Inflammatory response following injury, to some extent is beneficial as it protects the body from harmful stimuli. However, if the injury is not well treated, it can disturb blood circulation and lead to chronic inflammatory disease. Thus, numerous studies have investigated the benefits of non-steroidal anti-inflammatory drugs (NSAIDs) in preventing and treating EIMD. This kind of drugs work as COX enzymes inhibitor. When the enzymes are inhibited, generation of prostaglandins will be limited. Thus, swelling and soreness will be reduced (Windju, 2011). However, chronic use of NSAIDs may develop adverse effects.

1.2 Problem Statements

Aware of the adverse effects of NSAIDs such as stomach ulceration, kidney failure, liver damage and skin problems (Adams *et al.*, 1969; Rainsford and Velo, 1992), resulting people begin to look for natural medicine that possess anti-inflammatory benefit which they believed can replace or reduce the dependence on NSAIDs. Recently, numerous investigations have examined the effects of natural interventions on reducing the symptoms of EIMD, such as bioavailable curcumin (McFarlin *et al.*, 2016), New Zealand blueberries (McLeay *et al.*, 2012), tart cherry juice (Connolly *et al.*, 2006), Panax ginseng (Jung *et al.*, 2011), beetroot juice (Clifford *et al.*, 2016), black currant nectar (Hutchison *et al.*, 2016) and many others.

In addition to them, Nigella sativa (NS) seeds, is also widely reported to carry anti-inflammatory benefit. In Malaysia, we called it as *habbatussauda* but different countries have different names for it based on their languages such as; *kalonji* (India and Pakistan) *habbat al-barakah*, or 'the blessed seed' (Arabic), and *siah daneh* (Persian) (Goreja, 2003; Shabana *et al.*, 2012; Hajhashemi, 2004). The herb has been planted in many parts of the world such as Middle East, western Asia, northern Africa and Europe.

NS seeds are very popular in all over the world as it is broadly used in daily cooking, spice additive, bread flavor, as well as aromatic items (Ramadhan *et al.*, 2011). Since more than 3,000 years ago, it was extensively used by pharaoh's family to cure many diseases as it was found in the King Tutankhamun's tomb.

Continuous studies have been attempted to see the properties of NS. It is reported that NS also possess anti-inflammatory property. Thymoquinone (TQ), as one of its active component is believed to work as the prostaglandin inhibitor. (Parveen *et al.*, 2011). A lot of studies had investigated the effects of the herb on several common human models of inflammation such as rhinositis, encephalomyelitis, asthma, peritonitis, and arthritis (Salem, 2005; Cingi *et al.*, 2011; Balaha *et al.*, 2012). However, the researcher did not found any NS study investigating its effects on EIMD occurred in human, as a model of inflammation.

Based on numerous evidences that NS can suppress the prostaglandins generation, it seemed reasonable to expect that NS may attenuate muscle damage and inflammatory response after exercise. Consequently, the present team of researchers has carried out a preliminary study (first study) to examine the effects of a single one-off dose of NS on EIMD in human as an inflammation model. As the doses used in the literatures were varied, the first study aimed to examine the effective dose to enhance the recovery effectively following EIMD by giving different quantity of NS to each group to be taken only once (single one-off dose). The subjects were 33 youth football athletes who were being randomly divided into three different groups of supplementation, which were; NS 3000mg, NS 1500mg, and placebo. They had undergone 10 sets of 10 repetitions of counter-movement jump (CMJ) with 30 second intermittent rest between set, with squat start and landing to induce muscle damage. A set of tests were conducted to measure muscular power, soreness, tenderness, thigh circumference, range of motion (ROM), creatine kinase (CK) and lactate dehydrogenase (LDH) at pre-exercise, 30 minutes, one day, two days, three days and four days post-exercise. Right after the exercise, a single one-off of NS dose (either 3000mg or 1500mg) or placebo was administered.

The findings of the first study showed that EIMD carried the potential as an inflammation model as the changes pattern of EIMD markers suggested that the intensity of the exercise protocol implemented the study was enough to facilitate muscle damage and inflammation. However, the specific dose to enhance the recovery following EIMD was unable to be found because regardless of how much quantity of NS given, the single one-off dose was failed to affect all measured parameters.

Thus, the present study was initiated by the main problems and limitations found in the first study. In the first study, it was expected that a single one-off dose of NS would have some effects on recovery but no such effects had been found. The reason could be the anti-inflammatory component of NS, thymoquinone (TQ), possess speedy clearance and delayed absorption rate after ingested orally by rats as reported by Abdelwahab *et al.* (2013) and Alkharfy *et al.* (2015).

If the rate is not very different to human, a single one-off dose is insufficient to ensure the component is already entering the blood circulation and still remain in the blood. This is because the compound might be already cleared from blood as early as in 12 hours post-exercise. Thus, in the present study it was planned to give NS to the participants continuously from prior to the exercise bout until recovery period. It was expected that the effects of NS on muscle damage would be found if the NS had been ingested not only once but for longer and continuous period of time.

In the first study, there were very limited parameters of muscle damage and inflammation have been measured. To analyse deeper about the inflammation response including the inflammation time frame following EIMD, more blood markers are needed to be analysed. This includes neutrophils, macrophages and certain inflammatory cytokines. So, the present study added more inflammation markers to be analysed.

Simply put, the problems and limitations in the first study that initiated this present study were; i) the single one-off dose method used in the first study was failed to give any significant effects on recovery in all measured parameters, thus prophylactic and therapeutic interventions were used in the present study, ii) the parameters to measure muscle damage and inflammation following EIMD in the first study were limited, thus IL-6, IL-1 β , neutrophils and macrophages were added to the parameters.

1.3 Objectives of the study

1.3.1 Main objective

To determine the effectiveness of prophylactic and therapeutic interventions of NS supplementation in enhancing recovery following exercise.

1.3.2 Specific objectives

1.3.2(a) To investigate the prophylactic and therapeutic effects of NS on muscular power output.

1.3.2(b) To investigate the prophylactic and therapeutic effects of NS on muscle soreness and tenderness.

1.3.2(c) To investigate the prophylactic and therapeutic effects of NS on muscle circumference.

1.3.2(d) To investigate the prophylactic and therapeutic effects of NS on range of motion (ROM).

1.3.2(e) To investigate the prophylactic and therapeutic effects of NS on blood markers; i.e. creatine kinase (CK), lactate dehydrogenase (LDH), IL-6, IL-1β, neutrophils and macrophages.

1.4 Hypotheses

1.4.1 Main hypothesis

H_0 : There are no significant differences in the prophylactic and therapeutic effects of NS in attenuating muscle damage and inflammation following exercise.

H_A : There are significant differences in the prophylactic and therapeutic effects of NS in attenuating muscle damage and inflammation following exercise.

1.4.2 Specific hypotheses

1.4.2(a) **H_{0_1}** : There are no significant differences in the prophylactic and therapeutic effects of NS on muscular power output. **H_{A_1}** : There are significant differences in the prophylactic and therapeutic effects of NS on muscular power output.

1.4.2(b) **H_{0_2}** : There are no significant differences in the prophylactic and therapeutic effects of NS on muscle soreness measured using VAS and pain scale algometer.

H_{A_2} : There are significant differences in the prophylactic and therapeutic effects of NS on muscle soreness measured using VAS and pain scale algometer.

1.4.2(c) **H_{03}** : There are no significant differences in the prophylactic and therapeutic effects of NS on muscle circumference.

H_{A3} : There are significant differences in the prophylactic and therapeutic effects of NS on muscle circumference.

1.4.2(d) **H_{04}** : There are no significant differences in the prophylactic and therapeutic effects of NS on range of motion (ROM).

H_{A4} : There are significant differences in the prophylactic and therapeutic effects of NS on range of motion (ROM).

1.4.2(e) **H_{05}** : There are no significant differences in the prophylactic and therapeutic effects of NS on blood markers; CK, LDH, IL-6, IL-1β, neutrophils and macrophages.

H_{A5} : There are significant differences in the prophylactic and therapeutic effects of NS on blood markers; CK, LDH, IL-6, IL-1β, neutrophils and macrophages.

1.5 Significance and benefits of the study

The outcome of the present study may provide new evidence of NS effects in treating EIMD in human. In the literature, experimental exercise model of EIMD are not commonly classified as the inflammation models even though EIMD also carried inflammatory response following it. This study may open the opportunity to bring EIMD into the group of inflammation models as well. Hence, the study about EIMD and inflammation will be deeper and there will be more options in choosing the alternatives to treat inflammation following exercise.

In addition, this study is expected to provide more understanding on the effects of NS herb as natural medicine that has a potential to work as anti-inflammatory medication. The use of NS also can be broadened into the sports field for preventing and treating muscle inflammation following exercise.

There are quite a number of NS products in the markets but no clear dosage provided on the label and if there is, the provided dosage is not specific to certain uses. Therefore, the continuous study on this matter will determine the optimal dosage of NS for prevention and treatment of muscle sore and inflammation problems.

1.6 Definitions

1.6.1 Conceptual definitions

1.6.1(a) Delayed onset muscle soreness (DOMS)

The sensation of pain and stiffness in the muscles following unaccustomed, intense or prolonged exercises, which can arise from 24 to 72 hours after exercise, then fade away by five to seven days after exercise (Close *et al.*, 2005).

1.6.1(b) Exercise-induced muscle damage (EIMD)

Injury of muscles following unacquainted exercise, especially if the exercise involves a large amount of eccentric contractions (Clarkson and Hubal, 2002).

1.6.1(c) Inflammation

An immune system's response to infection and injury that leads to removal of offending factors and restoration of tissue structure and physiological function (Ricciotti and FitzGerald, 2011).

1.6.2 Operational definitions

1.6.2(a) Prophylactic intervention

An intervention designed as the prevention from EIMD, which is given two weeks before and during recovery of EIMD.

1.6.2(b) Therapeutic intervention

An intervention designed as the treatment of EIMD, which is given on the exercise day and during recovery of EIMD.

CHAPTER 2

LITERATURE REVIEW

2.1 Contents of the chapter

This chapter started with the explanations about EIMD, its causes and symptoms as well as the local conditions of muscles in the occurrence of EIMD. The explanations then were expanded to the relationship between inflammatory response and EIMD as there were many debates on the time course of the inflammatory responses following EIMD. Some studies reported inflammatory response occurs inevitably following EIMD but some other studies reported inflammatory response occurs if only when muscles was damaged in certain levels.

This chapter also explained literature reviews on nutritional strategies in preventing and attenuating EIMD. As NS is widely known to have anti-inflammatory properties, more explanation regarding the mechanisms of the anti-inflammatory activity of Nigella sativa and its promising effects on preventing and treating EIMD are reported in this chapter. It also includes the uses, background, constituents and toxicity of the herb. As this study was initiated from the limitations found in the first study by the same team of researchers, the reports of the first study are also included in this chapter.

2.2 Exercise-induced muscle damage (EIMD)

EIMD has been a topic of intense focus in exercise and sports science research for more than 30 years (Peake *et al.*, 2016). It usually occurs after unaccustomed or novel exercise, especially if the exercise involves a large amount of lengthening exercise (eccentric) (Clarkson and Hubal, 2002). EIMD is classified in type I muscle injury. It is based on the theories that type I muscle injury is distinguished by soreness which occurs at 24 to 48 hours after unaccustomed exercise and the soreness is well known as 'delayed onset muscle soreness' (DOMS) (Safran *et al*., 1989). As we already know, DOMS is one of the symptoms of EIMD, as stated in 2.21.

2.2.1 Symptoms of EIMD

There are a few temporary symptoms following EIMD as reported in the literatures such as swelling, soreness and stiffness, a decline in force production of muscle, an increase in passive tension, as well as a rise in muscle proteins in blood circulation (Peake *et al.*, 2005; Howatson and van Someren, 2008). The theories were supported later by Hyldahl and Hubal (2014), which reported in their study that the symptoms following EIMD are characterized by myofibrillar disruption, loss of muscle strength and power, DOMS, swelling, reduced range of motion of the affected limb, myocellular enzymes's systemic efflux and proteins such as CK and myoglobin or a combination of these.

More than 20 years ago, Gibala *et al.* (1995) reported in their study that there was a rise of disruptions of normal myofibrillar banding pattern at right after eccentric exercise and the statement was then supported by Yu *et al.* (2004) and Crameri *et al.* (2007), adding the disruption of Z disks and sarcomeres peaked between one and three days after exercise. Although many research papers focused on the association of EIMD with muscle fiber disruption, Nosaka (2008) later found that EIMD is highly associated with the connective tissues damage.

2.2.2 Experimental models of EIMD (muscle injury induced by exercise-employed experiments)

Previous studies reported that there were many experimental model of EIMD have been used. The experiments usually employed an exercise which involved more eccentric contractions rather than concentric contractions because it is very well-known that eccentric contractions can induce more damage to the muscles compared to the concentric contractions.

In the report written by Gordon and Palubinskas (2007), they summarized that there are five common experimental models of EIMD which included elbow flexor (EF), knee extensor (KE), downhill running (DHR), downward-stepping and prolonged-racing model. Among the five models listed, EF model is known to have highest intensity compared to other models. In the models which employ exercises such as EF and KE, isokinetic dynamometer is usually used to induce muscle injury using the constant angular velocity set by the researcher. Meanwhile, in the DHR model, running exercise was employed on a motor-driven treadmill at constant downhill grades varying from -5% up to -25% set by the researcher. Both of the exercises should be done in the lab or specific rooms that can place all the equipment needed, contrary to downward-stepping and prolonged-racing models which can be practically done on the field. In the downward-stepping model, the participants should step down with one leg either dominant or not dominant leg repetitively using the protocol designed by the researcher. In the prolonged-racing models, the usual exercises employed are marathon and ultra-marathon. Even though exercises such as downward-stepping and prolonged-racing are very practical to be done on the field, the velocities, angles, forces, work volume and a few more aspects cannot be standardized compared to EF and KE.

However, from our point of view, the experimental models should not be limited to the stated models only. There were many options in choosing a suitable experimental model, either creating a new model, using the model that has been widely used in the literatures. However, in the EIMD-related studies, the experimental models used should implement the exercise that has enough intensity to make sure the damage can be induced to the muscles so that we can see the difference in muscle damage parameters between the subjects with damaged muscles and the controlled subjects.

On the basis of the understandings, we implemented CMJ in our first study and keep implemented CMJ in this study as a model to induce damage to muscles. This model employed a squat stance in the both starting and landing positions in order to maximize eccentric contractions. In this model, the eccentric contractions occur when subjects bend down the hips in the beginning of jumping and landing positions. During the squat stance in the beginning of jumping and landing, hip flexion phase is controlled by the gluteus and hamstring, meanwhile knee flexion phase is controlled by eccentrically lengthened quadriceps muscles. During CMJ, concentric contractions occur when the subjects are in the flight phase, which employs explosive jump.

Our study also employed CMJ as the exercise model because it was proven in the first study that the intensity is sufficient to facilitate damage to the muscles. Apart from our study, there are many other researchers reported that they used this exercise model in their EIMD-related studies (Byrne and

Eston, 2002; Jakeman *et al.*, 2009; Clifford *et al.*, 2016; Marginson *et al.*, 2005).

2.2.3 Inflammatory response and EIMD

After performing unaccustomed or injurious training, disruption might happen to the structure of the muscle cells or tissues. A common response to this disruption, inflammatory process takes place by infiltration of inflammatory cells and proteins within the exercised muscle to repair and regenerate the injured muscle. Inflammatory cells mostly include neutrophils and macrophages, whereas inflammatory proteins include pro-inflammatory cytokines and cyclooxygenase-2 (COX2).

Within an hour following EIMD, numbers of neutrophil begin to elevate until 24 hours later. When neutrophils begin to decrease, the concentration of macrophages increases (Tidball, 2005). It is because in the beginning stage of muscle injury, neutrophils contribute larger than macrophages by damaging healthy regions of injured muscle fibers, adjacent uninjured muscle fibers or both, which may cause additional damage to the injured muscle. In this stage, restoration of normal structure and function of the muscle is temporarily impaired. This impairment might due to the reactive oxygen species (ROS) and certain cytokines (such as IL-1β, TGF- β1, TNF-α, and IFN-γ) released by neutrophils during phagocytosis.

However, in the next stage of muscle injury, macrophages concentration begins to increase to promote muscle repair and regeneration by removing damaged tissues. The regeneration of the muscle might be aided by certain cytokines (such as IL-6, IGF-1, PDGFs and LIF) released by macrophages during phagocytosis (Pizza, 2007).

In addition to neutrophils, macrophages and cytokines, COX2 also contribute in the generation of the inflammatory response, as a pro-inflammatory mediator in the arachidonic acid pathway that produces prostaglandins (Ricciotti and Fitzgerald, 2011). Prostaglandins (in particular to prostaglandin E_2) are very important in inflammatory process by sensitize pain receptors (Mense, 1993), inducing vasodilation, increasing vascular permeability, local blood flow, and body temperature (Leadbetter, 1995). The secretion of prostaglandins can cause free radicals production, which may increase vascular damage and toxicity in cells, thus worsening the injured tissues.

2.2.4 Time course of recovery following EIMD

According to Hyldahl and Hubal (2014), the alterations and oxidative stress occurred following EIMD could trigger a few types of cells located within the muscles to initiate the repair and remodeling of the muscles. It includes satellite cells as well as inflammatory, vascular and stromal cells. Satellite cell is known as muscle stem cells. The inflammatory cells are including neutrophils, macrophages, mast cells and T lymphocytes. The examples of vascular cells are pericytes and endothelial cells, meanwhile fibroblasts are the examples of stromal cells. Following EIMD, the cells interact with each other inside the extracellular matrix (ECM) of the damaged skeletal muscles. The graphical illustration of the cells that associate with muscle recovery and its time course is shown in Figure 2.1.

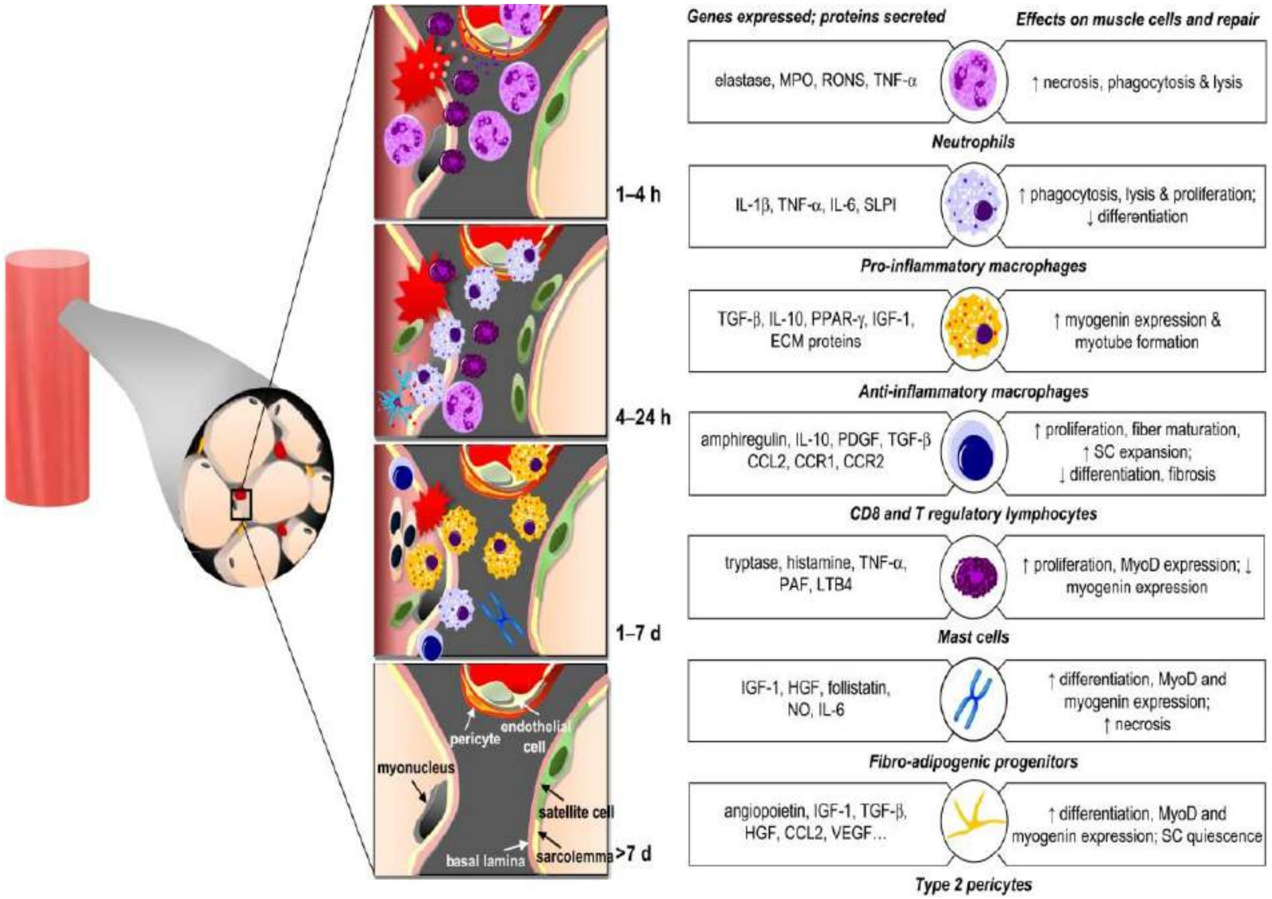

Figure 2.1 Cell types in the skeletal muscle that associate with muscle adaptation following exercise and its time course of recovery illustrated by Hyldahl and Hubal (2014)

Figure 2.1 shows the recovery period starts with the domination of neutrophils in the ECM near the damaged muscle to sweep away the cellular debris. This will initiate the inflammatory response of the pro-inflammatory and anti-inflammatory macrophages as well as cytokines within a day following EIMD. After a day, mast cells also permeate muscle tissue, releasing chemo-attractants and histamine. From four hours to a day following muscle damage, pro-inflammatory macrophages infiltrate the muscles and begin their jobs to secrete the pro-inflammatory cytokines, phagocytize the damaged muscle tissues and trigger the proliferation of myoblasts.

The pro-inflammatory macrophages then replaced by anti-inflammatory macrophages and lymphocytes after one day following muscle damage. These cells begin their actions to secrete anti-inflammatory cytokines, increase myoblasts proliferation and expand the satellite cell. After that, stromal cells are activated and myblasts differentiation is increased to support regeneration of new muscle fibers. As illustrated by Hyldahl and Hubal (2014), the ultrastructure of the muscle tissues then restored by seven days following muscle damage.

In addition to the studies focusing on the association between the interaction of the cells and the time needed to restore the muscle tissues, there are also many studies expanded the focus to the association between the symptoms (such as muscular strength) and the time needed to restore muscle functions after the damage. As reported by Crameri *et al.* (2007), there is a relation between the extent of loss of muscular strength following exercise and the time needed to restore the muscular strength. When muscular strength had declined by twenty percents right after exercise, it would be restored within two days following exercise.

However, Paulsen *et al.* (2010) found that when muscular strength had declined by 50% right after the first bout of eccentric exercise, it would be restored within seven days following exercise. It was then concluded by Damas *et al.* (2016) that there is a wide variations in the time course of changes in muscular strength, range of motion, swelling, DOMS as well as blood serum activity following eccentric exercise.

2.2.5 Is inflammation following EIMD good or bad to body?

Inflammation is an inevitable response of body to protect itself from injury, illness or infection. It is a part of our body's immune response and it acts by identifying and clearing harmful stimuli. If it is very beneficial, why it has to be prevented in some conditions? So, we work on the answers.

Firstly, it is true that it plays an important role in healing but it can also play a role in making an injury lasts longer than necessary, and if that so it will be harmful to the body. As having too much of a good thing is not always good, having too much inflammation also to some extend is not good because it can cause tissues destruction. It is due to the release of some enzymes will worsen the damaged of cells and tissues. In addition, it may cause too much of oedema or swelling which can be very serious when it occurs near the major organs such brain and heart. Too much swelling can disturb the blood circulation and this can possibly lead to death.

Secondly, as mentioned by Hobson (2017), even though inflammation is needed as body protector against infections, when the response is continuously activated for some times, it can be very harmful to the body such as what had happened in auto-immune disease like rheumatoid arthritis. The same theory goes to heart attack cause. It is believed that the accumulation of cholesterol could injured the walls of blood vessels, thus activate an inflammatory response that can cause blood clots in the heart.

Snyder (2016) also agreed that inflammation is crucial for tissue repairing and regeneration. However, chronic inflammation leads to many diseases such as atherosclerosis, asthma, arthritis, diabetes, cancer as well as blindness, and not to forget mental illness and possibly autism.

2.2.6 Indirect markers of EIMD and inflammation

There are two ways to perform analysis of muscle damage markers, which is either by examining direct markers or indirect markers. In animal, direct markers can be easily analysed directly and it has been widely done by attaching the anesthetizing animals to a fixed apparatus and electronically activate the muscle action. After the muscle has been damaged to certain extent, the animals are killed in few ways that has been set by the lab or organisation. The muscles needed for the analysis are dissected (Tiidus, 2007).

On the other hand, analysis of direct muscle damage markers in human studies cannot be performed easily because it is hardly getting ethical approval. However, it is possible to be done using biopsy protocol, whereby the small piece of muscle needed is removed for the analysis. Because of the problems and limitations in performing biopsy, researchers usually chose to perform analysis on indirect muscle damage markers such as MRI, blood proteins and enzyme activity, inflammatory response, strength loss, ROM, and soreness (Tiidus, 2007).

a. Magnetic Resonance Imaging (MRI)

MRI is very useful to assess the changes of whole muscle after eccentric exercise. This can identify the specific muscle damaged during eccentric exercise and the duration of the injury. MRI may provide explanation for functional loss of the muscle after eccentric exercise.

However, using MRI might be a bit costly. The intensity of MRI signal, which is called T2 relaxation time, is dependent on the amount of water in the tissue and it reflects edema (swelling) (Tiidus, 2007).

b. Blood proteins and enzyme activity

The most common parameters used in the studies related to EIMD are the analysis of blood proteins and enzymes activity levels after exercise to examine the appearance and the time course of muscle damage. Blood proteins are examined because they reflected what is released by the tissue and what is cleared from circulation. Few commonly examine proteins are: CK, LDH, myoglobin, troponin-I, myosin heavy chain (MHC), and β-glucuronidase (Tiidus, 2007).

c. Inflammatory response

Infiltration of neutrophils, macrophages and certain cytokines are the most common markers of inflammation response in muscle tissue such as after eccentric exercise, and it can be proven using direct and indirect evidence of inflammation. Direct evidence is examined using muscle biopsy procedure, which is said to have failure to use a control group and has many limitation. Malm *et al.* (2000) added that the biopsy procedure itself may injured the muscle and generate inflammation response, which may resulted in inaccuracy of the samples measured. While direct evidence of inflammation is