THE EFFECTS OF HYDROGEN-RICH WATER INGESTION ON THE SYMPTOMS OF ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE

ROS AMIZAH BINTI ABDULLAH

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by

ROS AMIZAH BINTI ABDULLAH

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LIST OF ABBREVIATIONS AND SYMBOLS

°	Degree
°C	Degree Celsius
%	Percent
1RM	One repetition maximum
ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAP	Biological antioxidant power
BMI	Body mass index
Ca^{2+}	Calcium ion
Mg^{2+}	Magnesium ion
CK	Creatine kinase
CO_2	Carbon dioxide
DHR	Downhill running
DNA	Deoxyribonucleic acid
DOMS	Delayed onset muscle soreness
dROM	Derivatives of reactive oxidative metabolites
EDTA	Ethylene diamine tetraacetic acid
EIMD	Exercised-induced muscle damage
ELISA	Enzym e-linked immunosorbent assay
g	Gram
$g \cdot day^{-1}$	Gram per day
GLM	General Linear Model
GSH	Glutathione
H	Hydrogen atom
H^+	Hydrogen ions

H_2	Hydrogen molecules
H_2O	Water
H_2O_2	Hydrogen peroxide
hr	Hour/hours
HSP	Heat shock protein
HSP70/72	Heat shock protein 70/72
HRP	Horseradish peroxidase
HRW	Hydrogen-rich Water
IL	Interleukin
IL-6	Interleukin-6
IU/L	International unit/litre
i.v.	Intra-venous
k	Kilo- (10^3)
kDa	kilodalton
$L \cdot day^{-1}$	Litre per day
lb	Pound
LSD	Lysergic acid diethylamide Least Significant Difference (test in statistics)
µ	Micro- (10^{-6})
µ	Microlitre
m	Milli- (10^{-3}) or metre
min	Minute
$min \cdot day^{-1}$	Minute per day
MIPS	Multi-ingredient performance supplements
METs	Metabolic equivalents
mL	Millilitre
mM	Millimolar
mol	Mole

mV	millivolt
N	Newton
$\bullet O^-$	oxide radical ion
•OH	hydroxyl radical
O_2	oxygen
O_2^-	superoxide anions
NSAID	Nonsteroidal anti-inflammatory drugs
PAR-Q	Physical Activity Readiness questionnaire
PCR	Polymerase chain reaction
ppb	Part per billion
PPO	Peak power output
RER	Respiratory exchange ratio
ROM	Range of motion
ROS	Reactive oxygen species
RPE	Ratings of perceived exertion
RT-PCR	Reverse transcriptase- polymerase chain reaction
rpm	Revolution per minute
s	Second
SD	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Package of Social Sciences
TNFα	tumor necrosis factor alpha
VAS	Visual analog scale
$\dot{V}O_2$	Oxygen consumption or uptake
$\dot{V}O_{2max}$	Maximal oxygen consumption

KESAN PENGAMBILAN AIR TINGGI KANDUNGAN HIDROGEN TERHADAP SIMPTOM-SIMPTOM KEROSAKAN OTOT AKIBAT SENAMAN EKSENTRIK

ABSTRAK

Kerosakan otot selepas senaman dicirikan oleh kemerosotan keupayaan penjanaan daya dan kesengalan otot tertunda (*delayed-onset muscle soreness*, DOMS). Sehingga hari ini, tiada kaedah terbaik untuk mengurangkan keadaan ini. Kebelakangan ini, manfaat air berhidrogen tinggi (HRW) telah dijelaskan melalui eksperimen dan keadaan penyakit klinikal. Kajian prospektif ini bertujuan untuk menilai kemampuan HRW, mengurangkan keterukan penurunan kekuatan dan DOMS selepas melakukan senaman dalam kalangan lelaki dewasa muda yang sihat dan juga aktif dalam aspek fizikal. Berdasarkan kaedah silang rawak, 11 orang lelaki dewasa (24 ± 4 tahun) menerima 1.7 L·hari $^{-1}$ sama ada HRW atau plasebo selama 14 hari, dengan tempoh rehat selama 28 hari antara ujian. Pada hari ketujuh rawatan, para peserta melakukan larian menuruni bukit (DHR) selama 30 minit di atas *treadmill* bermotor pada kelajuan bersamaan dengan 65% daripada pengambilan oksigen maksimum ($\dot{V}O_{2max}$) mereka dan kecerunan –15%. Penilaian mengenai DOMS dan ketenderan, julat pergerakan (*range of motion*, ROM) fleksi lutut, lilitan paha, satu kali pengulangan maksimum (1RM) kekuatan ekstensi lutut, serum kreatina kinase (CK), dan plasma *heat shock protein* 70 (HSP70) telah dijalankan sebelum dan diulang pada pelbagai titik masa (hari 0, 1, 2, 4 dan 7) selepas DHR. Perubahan pemboleh ubah pada parameter-parameter tersebut telah dibandingkan menggunakan langkah berulang dua arah ANOVA. Kenormalan data disahkan melalui ujian Shapiro-Wilk's pada *studentized residuals* ($p > 0.05$) dan data terpencil dikenal pasti berdasarkan nilai

studentized residuals lebih besar daripada ± 3. Kesferaan data telah disahkan oleh Ujian Mauchly. Analisis Greenhouse-Geisser digunakan apabila andaian kesferaan tidak dipenuhi. Kesan masa yang ketara diperhatikan dalam semua variabel kecuali lilitan paha. Terdapat peningkatan DOMS dan menurunkan ROM lutut dalam masa dua hari selepas DHR. Sementara itu, kehilangan kekuatan yang signifikan berterusan sehingga empat hari selepas bersenam. Serum CK meningkat pada hari pertama selepas DHR, manakala HSP70 pada hari kedua. Jelas sekali, HRW membantu mengurangkan DOMS pada 0 hari ($p \leq 0.045$) selepas DHR, serta mengurangkan tahap CK dan HSP70 pada hari keempat ($p = 0.043$) dan kedua selepas DHR. Sebaliknya tiada perbezaan yang signifikan diperhatikan antara rawatan bagi perubahan dalam ROM fleksi lutut, lilitan paha, kenyerian otot, dan 1RM kekuatan ekstensi lutut. Sebagai kesimpulan, HRW dapat membantu memperbaiki hasil yang tidak diingini dari senaman eksentrik seperti DOMS, serta pengaliran CK ke dalam peredaran darah dan rangsangan tekanan sistemik.

THE EFFECTS OF HYDROGEN-RICH WATER INGESTION ON THE SYMPTOMS OF ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE

ABSTRACT

Post-exercise muscle damage manifests as a decline in the force-generating ability and delayed-onset muscle soreness (DOMS). Until today, there is no single best method to alleviate these conditions. Recently, the beneficial effects of hydrogen-rich water (HRW) have been described in experimental and clinical disease conditions. This prospective research was aimed to evaluate the ability of HRW to reduce the severity of strength decline and DOMS post-exercise in healthy as well as physically active male youths. In a randomized crossover manner, 11 adult males (24 ± 4 years) received 1.7 $L \cdot day^{-1}$ of either HRW or placebo for 14 days, with a 28-day washout period between the trials. On the seventh day of the treatment, the participants undertook a 30-minute bout of downhill running (DHR) on a motorized treadmill at a speed corresponding to 65% of their maximal oxygen uptake ($\dot{V}O_{2max}$) and a slope of −15%. Assessments on DOMS and tenderness, knee-flexion range of motion (ROM), circumference of the mid-thigh, one-repetition maximum (1RM) knee extension strength, serum creatine kinase (CK), and plasma heat shock protein 70 (HSP70) were conducted before and repeated at various time points (Day 0, 1, 2, 4, and 7) post DHR. The changes in the physiological variables above were compared using a two-way repeated-measures ANOVA. The normality of the data was verified by Shapiro-Wilk's test on studentized residuals ($p > 0.05$), and the outliers were identified based on the studentized residuals for values greater than ± 3. The equal variances of the data were determined using Mauchly's test of sphericity. Greenhouse-Geisser correction was used when the assumption of sphericity was not met. Significant time effects were

observed in all variables except thigh circumference. There was an increase in DOMS and decline in knee-flexion ROM in the first two days post-DHR. Meanwhile, a significant amount of strength loss persisted until four days after exercise. Serum CK was elevated within the initial one day of DHR, while HSP70 two days. Evidently, HRW helped alleviate DOMS at 0 day post-exercise ($p \leq 0.045$), as well as reduce CK and HSP70 levels at day four ($p = 0.043$) and day two following DHR, respectively. On the other hand, significant trial-based differences were not noted in other measures. To conclude, HRW may help ameliorate certain undesirable outcomes of eccentric exercise like DOMS as well as the efflux of CK into circulation and systemic stress response.

CHAPTER 1 – INTRODUCTION

1.1 Background of Research

Hydrogen (H) is the smallest and third-most-abundant element on the Earth's surface, and approximately 90% of the visible universe is composed of hydrogen (Armaroli and Balzani, 2011). It consists of one proton and one unpaired electron, which makes it a free radical. The atomic hydrogen rarely exists on its own because the unpaired electron is nearly always bond with another electron to form a diatomic hydrogen molecule, which is known as molecular hydrogen (H_2). Molecular hydrogen is the most common form of hydrogen – it is a colorless, odorless, tasteless, non-toxic, non-metallic, lightweight gas – that stable with a neutral charge and hence it is not a free radical (Ohta, 2015).

The landmark study of Ohsawa *et al.* (2007) found that molecular hydrogen has antioxidant and antiapoptotic properties that could protect against cerebral ischemia-reperfusion injury by selectively reducing cytotoxic reactive oxygen species (ROS) and reactive nitrogen species (RNS). Since then, the therapeutic effects of molecular hydrogen have been reported in essentially all organs covering 31 disease categories that can be subdivided into 166 disease models including human diseases and pathophysiological conditions of plants, predominantly associated with oxidative stress and inflammatory insults (for Reviews, refer to Ichihara *et al.*, 2015; Nicolson *et al.*, 2016). Of all the 321 original studies profiled in the review of Ichihara *et al.* (2015), there are at least nine different routes of molecular hydrogen administration:

hydrogenated saline (for intraperitoneal injection or drip infusion), H_2-rich water (HRW) ingestion, H_2 gas inhalation, H_2-enriched solution, intestinal H_2 gas, H_2-enriched dialysis, H_2-rich water bath, instillation and H_2-rich tablet. The best modality of molecular hydrogen administration remains uncertain. However, apart from the hydrogenated saline, which is exclusively used in China, HRW ingestion has been the most popular method of molecular hydrogen administration due to its practicality (Ichihara *et al.*, 2015).

The clinical benefits of molecular hydrogen therapy have been explored in various human diseases such as type II diabetes mellitus (Kajiyama, Hasegawa, Asano, Hosoda, and Fukui, 2008), hemodialysis (Nakayama *et al.*, 2010; Nakayama *et al.*, 2017; Nakayama *et al.*, 2018), rheumatoid arthritis (Ishibashi et al., 2012), Parkinson's disease (Yoritaka *et al.*, 2013; Yoritaka *et al.*, 2018), muscular dystrophy and dermatomyositis (Ito et al., 2011). Molecular hydrogen therapy also has been applied in exercise and sports science research for its antioxidant properties (Aoki *et al.*, 2012; Kawamura *et al.*, 2016), buffering capacity (Ostojic and Stojanovic, 2014), and ergogenic potentials (Aoki *et al.*, 2012; Ostojic, 2012). It is shown that HRW ingestion reduces blood lactate level post ergometric cycling (Aoki *et al*., 2012), improves blood alkalinity indicator after treadmill running (Ostojic and Stojanovic, 2014), and can maintain peak power output following intermittent cycling (Da Ponte, GioVanelli, Nigris, and Lazzer, 2018). Besides, by supplementing molecular hydrogen therapy (H_2 tablet and H_2 pack) to traditional treatment, resulted in an improvement in the range of motion (ROM) and a reduction in plasma viscosity, and subsequently enhanced recovery of sports-related soft tissue injuries among athletes (Ostojic, Vukomanovic, Calleja-Gonzalez, and Hoffman, 2014).

It is known that ROS production significantly increased during physical exercise (Davies, Quintanilhat, Packert, and Brooks, 1982b), causing oxidative damage and inflammation of skeletal muscle. Numerous antioxidant supplements have been studied for their potentials in protecting against exercise-induced muscle damage (EIMD) during downhill running (DHR) (Herrlinger, Chirouzes, and Ceddia, 2015), repeated leg extension exercise (Bowtell *et al.*, 2011), and ergometric cycling (Jackson *et al.*, 2004; Khassaf *et al.*, 2003). However, the effectiveness of HRW as an antioxidant and its therapeutic potential in alleviating EIMD-associated symptoms such as muscle soreness and transient loss in muscle strength have not been examined. Knowledge of the effects of HRW in protecting against EIMD and enhancing the recovery of soreness and strength loss will be beneficial for individuals undertaking a series of physical work or exercise training when a rapid recovery between the workouts is necessary. Therefore, this project was designed to investigate the therapeutic potential of HRW ingestion on downhill running-induced muscle damage.

1.2 Problem Statement

As molecular hydrogen has proven to have antioxidant properties (Ohsawa *et al.*, 2007), it is plausible that it may protect muscle tissues from oxidative damage during physical exercise (Davies *et al*., 1982). Previous studies support the beneficial effects of the HRW ingestion on repeated sprint performance (Da Ponte *et al*., 2018), muscle fatigue (Aoki *et al*., 2012), and exercise-induced metabolic acidosis (Aoki *et al.*, 2012; Da Ponte *et al.*, 2018; Ostojic and Stojanovic, 2014). To date, we are not aware of any study specifically focused on examining the therapeutic effects of HRW ingestion in

alleviating the common symptoms of EIMD such as delayed-onset of muscle soreness (DOMS), muscle strength loss, increases in circulating creatine kinase (CK) and heat shock protein 70 (HSP70). The lack of human experimental trials in this area has provided us the impetus to investigate whether HRW ingestion could protect the human skeletal muscle against and enhance its recovery from EIMD.

1.3 Objectives of the Research

The primary objective of this study was to investigate the effects of HRW on eccentric exercise-induced muscle damage in healthy and physically-active human male participants. Specifically, this study examined the effects of 14 days of HRW ingestion (1.7 $L \cdot day^{-1}$) in ameliorating the adverse symptoms of muscle damage following a 30-minute bout of DHR by measuring 1) self-perceived muscle soreness and maximal muscle strength, 2) range of motion (ROM), muscle edema and muscle tenderness, and 3) common biomarkers of muscle damage, such as serum CK activities and plasma HSP70 response.

1.4 Hypothesis

We hypothesized that HRW ingestion would ameliorate muscle soreness and tenderness, the reduction in ROM, muscle edema, and strength loss in the lower limbs of the participants, as well as the rise in serum CK activity and plasma HSP70 during the recovery from a bout of muscle-damaging DHR.

1.5 Terminology Glossary

1.5.1 HRW Ingestion

Distilled water with a high content of dissolved hydrogen molecules (900 to 1103 ppb) was given orally to healthy and active young participants during the HRW trial in the present study. The purpose of HRW administration is not to treat any diseases but to obverse any effect of HRW ingestion on EIMD symptoms.

1.5.2 HRW Treatment

HRW is given to a treatment group within a certain period of time, whether taken orally (Da Ponte *et al.*, 2018; Yoritaka *et al.*, 2013) or as bath water *(*Kawamura *et al.*, 2016; Kawamura *et al*., 2018) to see its effects on the disease or symptom experienced by the subject. This effect can be seen by comparing treatment groups and placebo groups.

1.5.3 Hydrogen Therapy

Any form of molecular hydrogen (e.g., gas, liquid, tablet) given to participants in a long duration to treat any diseases or culture cells with disease conditions (Kang *et al.*, 2011; Nakayama, Kabayama, and Ito, 2016; Ostojic *et al.*, 2014; Yoritaka *et al.*, 2013).

CHAPTER 2 – LITERATURE REVIEW

2.1 Muscular System

Skeletal muscles are responsible for numerous physiological functions (Xia *et al.*, 2006), such as breathing and body movements. According to Xia *et al.* (2006), approximately 40% of a person's weight is accounted for by skeletal muscles – a group of striated muscles, which can be observed as light and dark bands microscopically. Myofibrils – the main component of muscular tissues – contain a basic structure of muscle contraction called sarcomere. Lieber and Fridén (2004) have mentioned that sarcomeres are the principal structures and contractile units in striated muscles. Also, their lengths are an important predictor of muscle damage. Sarcomeres are separated from each other by Z-discs, which are involved in mechano-sensation and nuclear signaling for muscle homeostasis. There are four types of protein filaments: actin-containing thin filaments, myosin-containing thick filaments, single protein-titin, and nebulin. Except for thick filaments, all others are attached to Z-discs (Clark *et al.*, 2002) and can extend toward the middle of the sarcomere.

The unique structure of a filament is crucial in effectuating muscle contractions, the latter of which is driven by changes in the structure of the myosin head that links actin-thin with myosin-thick filaments (Huxley, 1969; Huxley, 1974) to form cross-bridges. During muscle contraction, the myosin head pulls the thin filament towards the center of the sarcomere. This pulling action repeatedly occurs in the presence of adenosine triphosphate (ATP), which then causes the thin filaments to slide along the thick

filaments. The sliding of filaments results in the shortening of sarcomeres and hence, muscle fibers (Huxley and Hanson, 1959; Huxley, 1969). Evidently, this mechanism of muscle contraction will only occur in the presence of sufficient levels of ATP, calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (Huxley, 1969).

2.2 Muscle Damage

An individual need to use muscles to perform the movements in the daily activities. Movements that exceed the maximum capacities of muscles can cause muscular damage. In sports, athletes often experience muscle damage as a result of physical trauma, which is also known as muscle injury. Yu, Carlsson, and Thornel (2004) have reported that skeletal muscle damage is characterized by the disruption of the structure of a sarcomere. Specifically, there is a widening of the Z-discs. Sarcomere damage can be identified by the presence of (1) changes in the structure of its myofilaments under light or electron microscopy, as well as (2) elevated creatine kinase levels in the plasma (Armstrong, 1990). Muscle damage is usually associated with reduced muscle strength (Warren *et al.*, 2002), DOMS, enhanced plasma enzyme activity, as well as elevated levels of intracellular muscle proteins such as lactate dehydrogenase, creatine phosphokinase (Schwane, Johnson, Vandenakker, and Armstrong, 1983), myoglobin, and creatine kinase (Sorichter et al., 2001). Meanwhile, MacIntyre *et al.* (2000) have noted increases in neutrophil count owing to muscle damage after eccentric exercise.

In a review, Armstrong (1990) has proposed the sequence of events in muscle injury: initial, autogenetic, phagocytic, and regenerative phases. The initial event – which occurs in the muscle fibers – initiates the process of injury that is usually induced by

physical or metabolic factors. The initiating events result in the elevation of intracellular Ca^{2+} (Ca^{2+} overload phase) owing to the disruption of Ca^{2+} homeostasis in the muscle fibers. In turn, several intrinsic degradative pathways are activated in the muscle fibers. The next phase of injury involves Ca^{2+}-activated degradative autogenetic mechanisms at the site of the injury. These consist of the phospholipase A2 (PLA2) cascade (which generates acid leukotrienes, prostaglandins, and arachidonic acid), lysosomal proteases, and Ca^{2+}-activated proteases. The elevation of intracellular Ca^{2+} also interrupts the normal respiration of mitochondria, thereby causing sarcomeric contraction. These autogenetic processes initiate the breakdown of the protein and lipid structures in the injured cells following the penetration of phagocytic cells into the damaged tissues. The phagocytic stage starts when the macrophages produce substances such as lysosomal proteases and free O_2 radicals, which cause the rapid breakdown of damaged muscle fibers. This stage reaches a peak within several days after exercise. Finally, the injured cells undergo healing, during which the functions of the myofibers are completely restored.

2.3 Exercise-induced Muscle Damage (EIMD)

One of the factors that trigger muscle damage is physical exercise. While numerous studies have been conducted to identify the flexibility of human skeletal muscle during physical activity, the actual mechanism behind the EIMD is yet to be discovered. One of the first research on EIMD has been performed by Hough in the 1900s. In his ergographic experiments, Hough (1902) found that muscle soreness (a painful sensation) occurred in untrained muscles. This sensation of soreness is produced by the movement. To date, a large number of studies have been conducted on EIMD, and

various methods used to induce muscle damage. One of the common methods is the incorporation of eccentric muscular contractions (Armstrong, 1990). Meanwhile, Newham *et al.* (1983b) have proven that eccentric contractions cause more damage than concentric contractions. Eccentric exercise is one form of exercise that is performed unaccustomedly, the results of which are muscle soreness and stiffness in the subsequent days (Proske and Morgan, 2001).

DHR is a type of eccentric exercise that has been used to induce muscle damage in several studies (Byrne *et al.*, 1985; Donnelly *et al.*, 1988; Donnelly, Maughan, and Whiting, 1990; Kingsley *et al.*, 2006; Lin *et al.*, 2016; Ormsbee *et al.*, 2015; Peschek *et al.*, 2013; Silva *et al.*, 2011; Sorichter *et al.*, 2001). This is because DHR induces more eccentric contractions of the knee extensors (thereby leading to more tissue damage) as compared with level- or uphill-running (Malm *et al*., 2004).

EIMD manifests as an increase in muscle soreness, as well as a decline in muscle strength and range of motion for several days post-exercise (Chen *et al.*, 2009; Clarkson, Nosaka, and Braun, 1992; Hyldahl and Hubal, 2014; Sousa, Teixeira, and Soares, 2014; Torres *et al.*, 2012). EIMD also characterized by the temporary disruption of the myofibrillar ultrastructure, in addition to the systemic effluxes of myocellular enzymes and proteins such as CK and myoglobin (Hyldahl and Hubal, 2014). As per Cheung, Hume, and Maxwelf (2003), the intensity of pain may increase for as long as 5 to 7 days post-exercise.

2.4 Evidence of Muscle Damage

The symptoms of muscle damage are commonly used as indirect markers of muscle damage. Measurements of the muscle damage are focused on the functional evidence of muscle damage, especially soreness, edema, loss of strength, and reduction in the range of motion (Warren, Lowe, and Armstrong, 1999). In addition, biochemical analyses of bodily fluids constitute important parameters of muscle damage after eccentric exercise. According to Warren, Lowe, and Armstrong (1999), 52% of human studies have used blood levels of CK, myoglobin, LDH, and glutamic-oxaloacetictransaminase as markers of eccentric contraction-induced muscle damage.

2.4.1 Muscle Soreness

As mentioned, one of the main symptoms of EIMD is muscle soreness. In their review, Warren, Lowe, and Armstrong (1999) reported that 73% of human muscle injury studies have employed muscle soreness as a variable of muscle damage. Meanwhile, Cleak and Eston (1992) have categorized muscle soreness into two: immediate-onset and delayed-onset. The former may be due to the effects of biochemical end-products of metabolism on the free nerve endings (Asmussen, 1956; Friden, 1984). This pain is short-lived and disappears when the activity stops. In contrast, DOMS usually appears around eight hours after exercise and peaks 24 to 48 hours post-exercise. This pain usually lasts up to 96 hours after exercise, following which the muscles fully recover in almost all cases (Clarkson, Nosaka, and Braun, 1992). Also, the severity of DOMS depends on the type and duration of the exercise. As a side note, DOMS has regularly

been discussed in research on EIMD. It relates to the feeling of muscle pain and tenderness when the muscle is contracting, or when pressure is applied to the muscle (Armstrong, 1984; Cheung, Hume, and Maxwelf, 2003). Other signs of DOMS include dullness, stiffness, swelling, and reduction of muscle strength (Abraham, 1977; Newham *et al*., 1983b).

As DOMS impairs an athlete's ability to perform physical activities, it has become a topic of interest among exercise professionals. Nevertheless, the exact causes of DOMS and the mechanisms that trigger it at the cellular level are still unknown. Three theories have been proposed to explain the said mechanisms, namely the mechanical trauma, muscle damage, and inflammation theories. Furthermore, a model that integrated these theories has been explained by several researchers (Armstrong, 1984; Cheung, Hume, and Maxwelf, 2003), as shown in Figure 2.1 below.

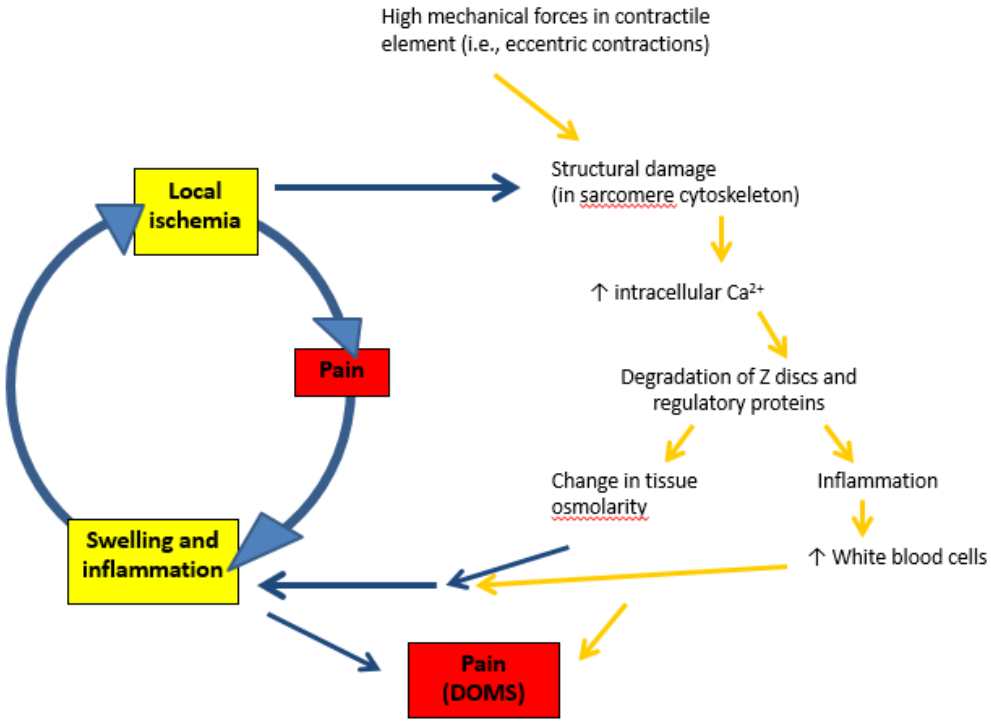

Figure 2.1 An integrated model to explain DOMS (Armstrong, 1984; Cheung, Hume, and Maxwelf, 2003)

Figure 2.1 illustrates a comprehensive model of DOMS. High-impact eccentric exercises entail unusual muscle contractions which alter the structures of proteins in the muscle fibers. Such damages usually occur at the Z-lines/ discs of the sarcomeres and in the connective tissues at the myotendinous junctions (Cheung, Hume, and Maxwelf, 2003). Another mechanism of DOMS involves damage to the sarcolemma of the myocytes, which eventually damages the sarcomeres. The former interferes with the production of ATP so that Ca^{2+} cannot be transferred to the sarcoplasmic reticulum for storage. From there, Ca^{2+} ions accumulate in the cells and hence, disrupt the structure of the sarcomere, eventually leading to tissue necrosis (Armstrong, 1984; Overgaard *et al.*, 2004).

On another note, the inflammation theory has been explained by Cheung, Hume, and Maxwelf (2003). Accordingly, damaged muscles cause intracellular enzymes such as CK to leak into the plasma and induce the accumulation of inflammatory cells at the site of injury. In such conditions, the blood vessels will dilate, and fluid will flow into the muscle tissues, thereby resulting in muscle edema. Debris, which contain dead cells and other components (such as histamine and potassium) accumulate in the muscles as well (Cheung, Hume, and Maxwelf, 2003). All these events increase the amount of pressure on the tissue, thus stimulating the pain nerve surrounding the area.

Several methods have been used to measure the severity of muscle pain, among which the Visual Analog Scale (VAS) is the most preferred. As reported by Warren, Lowe, and Armstrong (1999), 63% of studies on muscle injuries have utilized VAS, or

numerical scale, to measure muscle soreness. VAS is a tool that consists of a 100-mm line with graduations ranging from “least possible pain” on the left and “worst possible pain” on the right (Bijur, Silver, and Gallagher, 2001). Table 2.1 summarizes the studies that have employed VAS to measure muscle soreness at rest and following eccentric exercise (primarily focused on DHR).

Table 2.1 Muscle soreness at rest and following eccentric exercise (primary focus on DHR) as measured by using VAS.

Reference	Objective	Participants	Exercise Protocol	Muscle Soreness, VAS Score (mm)						
				Pre	Day-0	Day-1	Day-2	Day-3	Day-4	Day-7
Cleary *et al.* (2005)	To determine whether heat stress (thermoregulation) during exercise adversely affected muscle injury and caused DOMS.	10 healthy males.	60 min treadmill-walking and 40 min DHR (–12˚ grade).	0.0	18.6	37.9	32.2	14.6	3.7	–
Cleary, Sitler, and Kendrick (2006)	To identify the effects of dehydration on 5 physiological characteristics of DOMS in normothermic men after eccentric exercise.	10 healthy males.	45 min treadmill-walking and 40 min DHR (–12˚ grade).	2.8	28.0	56.9	45.7	24.5	11.7	–
Chen *et al.* (2009)	To compare three different intensities of DHR (70%, 80%, and 90% $\dot{V}O_{2max}$) of DHR for post-DHR changes in running economy in untrained individuals.	15 untrained adult males.	30 min DHR (–16% grade).	0.0	29.2	113.9	119.3	89.3	41.6	17.9
Welsh, Allen, and Byrnes (2014)	To determine if an eccentrically-dominated task (DHR) produces changes in plasma MMP-9 or TIMP-1 level. To examine the relationship between MMP-9/TIMP-1 levels and indirect indicators of muscle damage.	12 sedentary subjects; 9 subjects with history of concentrically-biased training.	30 min DHR (–17.5% grade). Speed: 70% of heart rate of APMHR.	4.8	30.0	41.0	43.3	–	15.0	5.0

Table 2.1 (Continued)

Reference	Objective	Participants	Exercise Protocol	Muscle Soreness, VAS Score (mm)						
				Pre	Day-0	Day-1	Day-2	Day-3	Day-4	Day-7
Ormsbee *et al.* (2015)	To determine the impact of MIPS (NO-Shotgun®) pre-loaded 4 weeks prior to a single bout of DHR on muscle soreness and performance.	20 healthy, male endurance-trained runners.	60 min DHR (–5% grade).	55.3	74.1	114.6	86.6	74.4	–	–
Mickleborough *et al.* (2015)	To evaluate the effects of PCSO-524® – a marine oil lipid and n-3 LC PUFA blend (derived from New Zealand green-lipped mussel, *Perna canaliculus*) on markers of muscle damage and inflammation following muscle-damaging exercise in untrained men.	32 untrained males.	20 min DHR (–16% grade). Speed: 70% of $\dot{V}O_{2max}$.	14	–	44	48	39	30	–
Lin *et al.* (2016)	To determine whether panax ginseng and danshen supplementation exerted protective effects on the vasculature following eccentric exercise.	24 young, healthy males.	30 min DHR (–10˚ grade). Speed at 75% of $\dot{V}O_{2max}$.	39	70	72	55	35	–	–

Table 2.1 (Continued).

Reference	Objective	Participants	Exercise Protocol	Muscle Soreness, VAS Score (mm)						
				Pre	**Day-0**	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-7**
McFarlin *et al.* (2016)	To determine the effects of oral curcumin supplementation (Longvida® 400 mg/day) on muscle and ADL soreness, CK, and inflammatory cytokines (TNF-α, IL-6, IL-8, IL-10) following EMID (eccentric-only dual-leg press exercise).	28 healthy subjects.	6 sets of 10 leg-press repetitions.	2	–	55	65	30	10	–
Maeo *et al.* (2017)	To investigate whether a 5-min prior bout of downhill walking protected against muscle damage induced by a 40-min subsequent bout of downhill walking.	36 young, healthy men.	Uphill and 40 min downhill walking (–28% grade).	0	–	70	75	45	–	–
Ely *et al.* (2017)	To determine the influence of antihistamines on post-exercise blood flow, inflammation, muscle damage, and DOMS in a model of moderate EIMD.	20 healthy volunteers (7 female, 13 male).	45 min DHR (–10% grade).	35	53.7 57.5 (6-h)	61.3	55	40	–	–
Oosthuyse and Bosch (2017)	To determine whether the response of serum CK and perception of DOMS	15 eumenorrheic women and 6 men (young, healthy,	20 min DHR (–10% grade).	6.3	43.8	50.0	43.8	25.0	–	–

following a bout of DHR is influenced by gender.	and not taking medications).	Speed: 9 km·h^{-1}.

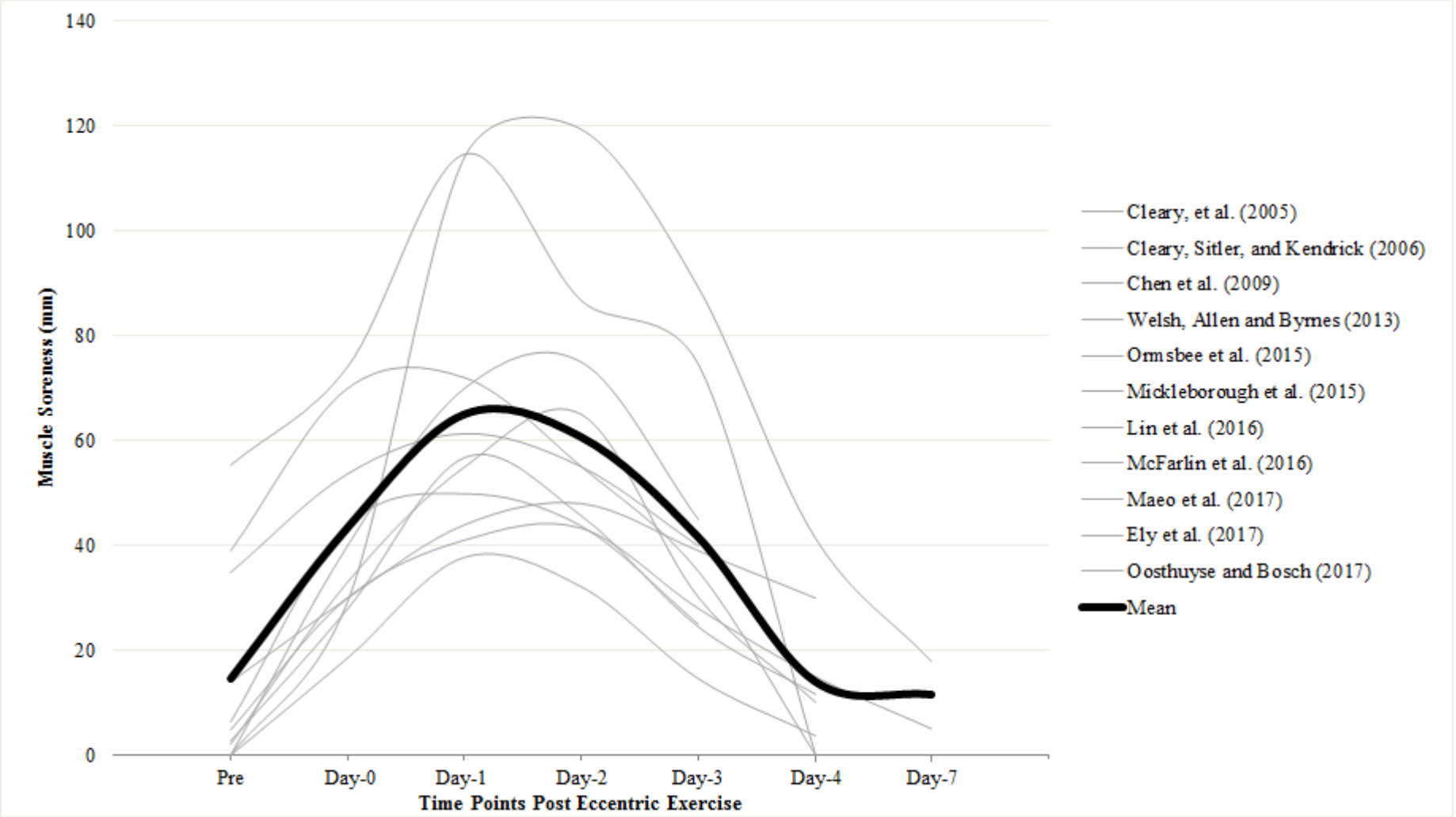

Figure 2.2 Summary of studies on muscle soreness as a result of eccentric exercise–induced muscle damage.

2.4.2 Muscle Edema

One of the post-exercise symptoms that reflect muscle damage is swelling (edema) of the affected region. Bobbert, Hollander, and Huijing (1986) have hypothesized that muscle edema following exercise was caused by Z-lines/discs interruptions, which led to the formation of protein-bound ions that interfered with the osmotic pressure in the tissues. Circumferences of muscle groups (anthropometry) are one of the methods for measuring edema. Accordingly, several studies have shown that there was an increase in this quantity, especially on day four to five post-exercise (Howell, Chleboun, and Conatser, 1993; Madden *et al*., 2011; Cleary, Sitler, and Kendrick, 2006; Howell *et al.*, 1985).

As per Cleary, Sitler, and Kendrick (2006), there was a significant bilateral increase in the distal thigh circumference 0.5 and 24 hours post-eccentric exercise (relative to pre-exercise). Since exercised muscles require more oxygen and glucose, there is increased removal of metabolic wastes owing to metabolic demand. In turn, vascular perfusion of the muscles is enhanced, thereby increasing the circumferences of the muscle groups half an hour post-exercise.

2.4.3 Range of Motion (ROM)

ROM – the maximum potential flexion or extension of a specific joint – is a variable for assessing the ability of a muscle to shorten when the limb is flexed to an angle (Clarkson, Nosaka, and Braun, 1992). Accordingly, several studies have used ROM to measure the extent of muscle damage after eccentric exercise (Tokmakidis *et al.*, 2003;

Madden *et al.*, 2011; Rawson, Gunn, and Clarkson, 2001). According to Warren *et al.* (1999), 19% of human studies have employed ROM as one of the methods to measure the severity of muscle damage.

Clarkson, Nosaka, and Braun (1992) have demonstrated a decline in the passive ROM of the elbow flexors subjected to eccentric free-weight loadings. Immediately after the exercise, most subjects could not flex or bend their arms completely because full ROM recovery was only completed within 10 days, even though it began within 24 hours. The decline in ROM was believed to be correlated with the extent of muscle edema. According to Howell *et al.* (1985), alterations in the resting ROM occurred concurrently with muscle edema 48 hours post-exercise. Thus, it has been suggested that the ROM was restricted by edema of the perimuscular connective tissue.

2.4.4 Muscle Strength

Strength is the ability of muscles to oppose a resisting force. Prolonged loss of muscle strength following the performance of eccentric exercise serves as an important indicator of the extent of EIMD. In further detail, 50% of human studies have employed maximal voluntary contraction torque to measure the severity of muscle strength loss (Warren, Lowe, and Armstrong, 1999). Also, it was found that 10 to 30% of muscle strength loss occurred after DHR (Eston *et al*., 2000).

Warren *et al.* (2002) have proposed the three mechanisms of muscle strength loss, namely (1) destruction of force-generating and/or force-transmitting structures; (2)

failure to maintain the intactness of the structures of power generation; as well as (3) frank loss of force-generating and/or force-transmitting structures.

One-repetition maximum (1RM) testing is the common method for measuring muscle strength and has been employed in the prescription of training programs for athletic trainers and strength coaches (Reynolds, Gordon, and Robergs, 2006). It is also a suitable method for use in resistance exercises that involve eccentric contractions (Rawson, Gunn, and Clarkson, 2001; Tokmakidis *et al.*, 2003; Welsh, Allen, and Byrnes, 2014). In general, the subject is required to perform muscular contractions until a maximum weight can be lifted once. However, there should be no more than 6 repetitions (Welsh, Allen, and Byrnes, 2014; Hutchins, 2010). Table 4.2 shows the list of studies that measure the loss of muscle strength following eccentric exercise.

Table 2.2 Studies on percentage of relative maximal muscle strength following eccentric exercise.

Reference	Objective	Participants	Exercise Protocol	Relative Maximal Muscle Strength (%)						
				Pre	Day-0	Day-1	Day-2	Day-3	Day-4	Day-7
Donnelly, Maughan, and Whiting (1990)	To test the effects of ibuprofen on muscle soreness, muscle weakness, and muscle damage as reflected by changes in serum enzymatic activities.	32 healthy, untrained males.	45 min DHR.	100	96.5 (6-h)	96.5	98.2	100	–	–
Eston *et al.* (1996)	To examine the effects of a prior bout of maximal isokinetic eccentric exercise on DOMS, strength loss, and plasma CK levels following DHR.	10 males.	40 min DHR (–10% grade).	100	79.3	–	86.0	–	93.0	94.0
Tokmakidis *et al.* (2003)	To examine the effects of ibuprofen on DOMS, indirect markers of muscle damage, and muscle performance.	19 healthy participants.	Isotonic eccentric protocol on the universal leg curl machine (bilateral).	100	68.6	83.3	88.2	–	–	–
	To examine the changes in markers of muscle damage and running economy for 5 days after DHR.	10 young men.	30 min DHR (–15% grade).	100	78.7 (1-h)	85.3	87.7	89.1	92.8	98.0

Table 2.2 (Continued).

Reference	Objective	Participants	Exercise Protocol	Relative Maximal Muscle Strength (%)						
				Pre	Day-0	Day-1	Day-2	Day-3	Day-4	Day-7
Chen *et al.* (2009)	To compare three different intensities (70%, 80%, and 90% $\dot{V}O_{2max}$) of DHR for post-DHR changes in running economy in untrained individuals.	15 adult males who have not participated in resistance and endurance training or recreational sport activities.	30 min DHR (–16% grade).	100	72.5	73.8	77.6	77.9	80.6	82.9
Welsh, Allen, and Byrnes (2014)	To determine if an eccentrically-dominated task (DHR) produces changes in plasma MMP-9 or TIMP-1 level and to examine the relationship between MMP-9/TIMP-1 levels and indirect indicators of muscle damage.	12 sedentary subjects; 9 subjects with history of concentrically-biased training.	30 min DHR (–17.5% grade). Speed: 70% of heart rate of APMHR.	100	92.0	94.0	95.0	–	98.0	102.0
Herrlinger, Chirouzes, and Ceddia (2015)	To examine the effects of a proprietary polyphenolic blend (which contained catechins and theaflavins) on exercise performance and recovery following eccentric exercise.	37 young, male participants.	40 min DHR (–10% grade).	100	–	93.0	91.8	–	93.8	–

Table 2.2 (Continued).

Reference	Objective	Participants	Exercise Protocol	Relative Maximal Muscle Strength (%)						
				Pre	**Day-0**	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-7**
Mickleborough *et al.* (2015)	To evaluate the effects of PCSO-524® – a marine oil lipid and n-3 LC PUFA blend (derived from New Zealand green-lipped mussel, *Perna canaliculus*) on markers of muscle damage and inflammation following muscle-damaging exercise in untrained men.	32 untrained males.	20 min DHR (–16% grade). Speed: 70% of $\dot{V}O_{2max}$.	100	–	91.2	90.7	92.8	102.2	–
Ely *et al.* (2017)	To determine the influence of antihistamines on post-exercise blood flow, inflammation, muscle damage, and DOMS in a model of moderate EIMD.	20 healthy volunteers (7 female, 13 male).	45 min DHR (–10% grade).	100	89.1 (6-h)	80.3	84.7	91.3	–	–
Maeo *et al.* (2017)	To investigate whether a 5-min prior bout of downhill walking protected against muscle damage induced by a 40-min subsequent bout of downhill walk.	36 young, healthy men.	40 min downhill walking (–28% grade).	100	–	80.0	86.7	90.0	–	–

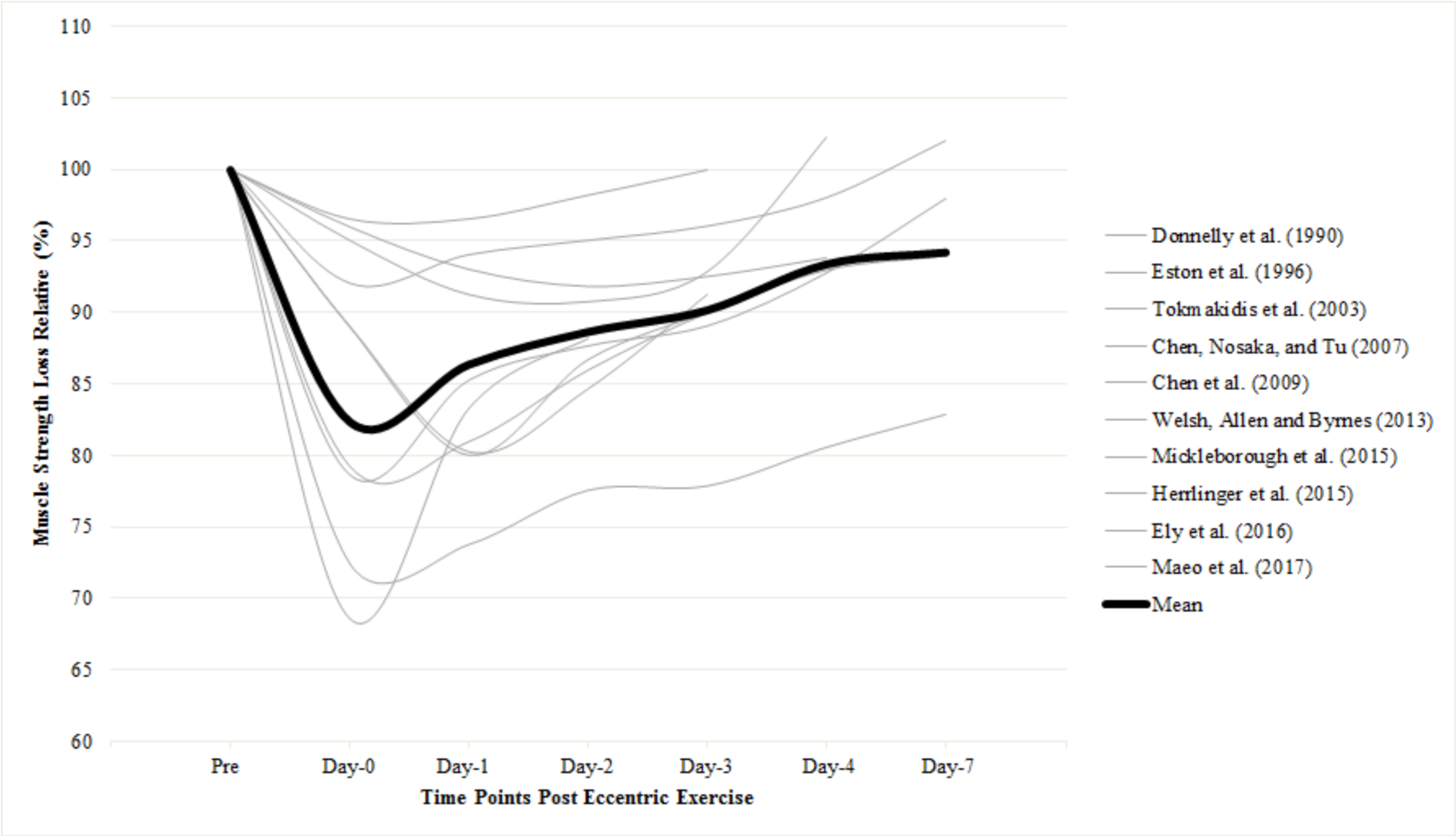

Figure 2.3 Summary of studies on muscle strength loss as a result of eccentric exercise–induced muscle damage.