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The effects of intermittent exposure to hyperbaric oxygen for the treatment of an acute soft tissue injury

by

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We accept this thesis as conforming
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ABSTRACT

This study examined the effects of intermittent exposure to hyperbaric oxygen therapy (HBO) for the treatment of delayed onset muscle soreness (DOMS). It is apparent in the literature that a great deal of controversy exists in using this form of therapy to treat tissue injuries. It was hypothesized that subjects exposed to hyperbaric oxygen would recover from DOMS faster than subjects exposed to normoxic air. Sixteen sedentary, female university students participated in the study and were randomly assigned to either an experimental or control group. All subjects performed 300 maximal voluntary eccentric contractions (30 sets of 10 repetitions/minute) of their non-dominant leg (110° - 35° of knee flexion) at a slow speed (30° per second) on the KinCom Dynamometer, to elicit muscle damage and injury. HBO treatments consisted of 100% oxygen for 60 minutes at 2.0 ATA while the control group received 21% oxygen at 1.2 ATA for the same amount of time. Both groups received treatment immediately after the induction of DOMS and each day after for a period of 4 days [day 2 post-exercise thru day 5 post-exercise]. Dependent variables (perceived muscle soreness, isokinetic strength, quadriceps circumference, creatine kinase (CK), interleukin-6 (IL-6) and malondialdehyde (MDA) were assessed baseline (pre-exercise, day 1), 4 hours post-exercise (day 2), 24 hours post-exercise (day 3), 48 hours post-exercise (day 4) and 72 hours post-exercise (day 5). MRI [T2 relaxation time/STIR]) was assessed baseline (day 1), 24 hours post-exercise (day 3) and 72 hours post-exercise (day 5). Isokinetic strength ($p<0.05$) and perceived soreness ($p<0.05$) indicated significance for injury to the quadricep muscle for both groups but no difference was seen between groups ($p=0.102$, $p=0.571$ respectively). Quadricep circumference was measured at the 10 and 20 cm reference point above the superior portion of the patella. The 10cm girth measurement indicated significance ($p<0.05$) for muscle injury but there was no difference between groups ($p=0.815$); 20 cm measurement showed no significance ($p<0.05$) for both within and between groups ($p=0.677$). No significance was evident for serum CK ($p<0.05$), both within and between groups ($p=0.647$). MDA analysis revealed no significance ($p<0.05$) both within and between groups ($p=0.580$). Analysis of IL-6 demonstrated no significance

($p<0.05$) for both within and between groups ($p=0.111$). Finally, MRI analysis for T2 weighted imaging of the rectus femoris, vastus medius and vastus lateralis showed no statistical significance ($p<0.05$) between groups for treatment effects ($p=0.800$, $p=0.361$, and $p=0.806$ respectively). Similarly, analysis of the STIR images indicated no statistical significance ($p<0.05$) for the same three muscles ($p=0.796$, $p=0.580$, and $p=0.265$ respectively). The findings of this study suggest that hyperbaric oxygen therapy was not effective in the treatment of exercise-induced muscle injury as indicated by the markers evaluated.

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DEDICATION

One individual who played an enormous part in my life and educational pursuits was my mother whose laughter, love and continuous support made a world of difference in my life. This is for you mom!

PREFACE

During this past decade, society has seen a growing number of individuals participating in sport and recreational activities. However, the number of injuries as a result of this increase in activity has also risen. Unfortunately, a primary cost related to injury recovery is the time-lost from participating in and resuming normal functional activity. This has compelled health care professionals to seek more efficient and effective therapeutic interventions in treating such injuries. Hyperbaric oxygen therapy may serve to provide a means of therapy to facilitate a speedier resumption to pre-injury activity levels as well as improve both the short and long-term prognosis of the injury.

Although a growing interest in sports and exercise medicine is becoming evident in the literature, the use of hyperbaric oxygen as an intervention in this field has been controversial. To date, numerous professional athletic teams, ranging from hockey (NHL), football (NFL), basketball (NBA) and soccer (MLS, European League), utilize and rely on the use of hyperbaric oxygen as adjuvant therapy for numerous sports-related injuries acquired from playing competitive sports. However, to date, there is a paucity of research on the application benefits of hyperbaric therapy and sports injuries. Further research needs to be conducted suggesting and validating the significant effects of this treatment modality and further grounding its importance in sports and exercise medicine.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction to Hyperbaric Oxygen

Although the roots of modern hyperbaric oxygen therapy date back three centuries, it is only in the last few decades that the scientific foundation has been laid recognizing the benefits of inspiring 100% oxygen at greater-than-ambient pressures [186]. Hyperbaric oxygen has been used in a variety of medical conditions and clinical settings but this form of therapy to treat soft tissue injuries remains a controversial issue.

Hyperbaric oxygen (HBO) therapy refers to the therapeutic procedure where patients inspire 100% oxygen while their entire bodies are subjected to pressures greater than ambient barometric pressure at sea level (the patient encounters pressures greater than one atmosphere absolute [1 ATA] or 760 mmHg). Oxygen is administered at a pressure above normal atmospheric to increase oxygen delivery to ischemic or hypoxic tissues. Barometric pressure changes during hyperbaric oxygen therapy are often expressed in multiples of atmospheric absolute (ATA) [3]. A change of 1 ATA is equivalent to 14.7 pounds per square inch (PSI), 760 mmHg or 33 feet of seawater (FSW) [3]. Therefore, an individual compressed to 2 or 3 ATA is equivalent to being 33-66 feet below the surface of the ocean [Table 1].

Gaining medical reputation in the last 35 years, the efficacy of HBO therapy has widely been accepted and utilized as the primary treatment of decompression sickness (illness resulting from rapid changes in pressure by divers or aviators), air embolisms (introduction of air into the circulation system, often involuntarily by medical professionals) and carbon monoxide poisoning. It has also been used as a successful adjunct in crush injuries, compartment syndromes, burns, traumatic ischemias, refractory wounds, necrotizing soft tissue infections, osteomyelitis, radiation tissue damage, compromised skin grafts and flaps and cases of extreme blood loss [3,9,34-35] [Table 2].

The principle of hyperbaric therapy is based on the following two components: 1) the *physiologic effects* of hyperoxemia (increase in partial pressure of oxygen dissolved in arterial blood) and 2) the *mechanical effects* of increased pressure [3,39]. Through these two components, enough oxygen is dissolved in the plasma to meet the metabolic needs of injured tissue. This is of extreme importance in soft tissue injuries where the environment is hypoxic. To date, hyperbaric oxygen has been recognized as a significant adjunct in the treatment of a variety of wounds. Ample evidence exists that suggests inspiration of oxygen above 21% to injured body tissue can have numerous beneficial effects [5-13]. It has been demonstrated that increasing the partial pressure of oxygen can counter local tissue hypoxia (under-oxygenation of tissue) [5-9], promote peripheral vasoconstriction [5-9], decrease blood flow to the area of insult [5,8-11], promote healing of damaged tissue and prevent infection by inhibition of the growth of anaerobic microorganisms [7,11-13].

The application of hyperbaric oxygen (HBO) for the treatment of sports injuries has recently been grounded in the scientific literature as a modality of therapy. However, the concept of using compressed gas for medical purposes has a rich history, with the origins and development of hyperbaric medicine being closely tied to the history of diving medicine [9, 31-32] [Table 3]. It was noticed that residents living at high altitudes had wounds that healed more slowly than at sea level as compared to people living in undersea habitats at hyperbaric pressures (deep sea divers) whose wounds healed faster at the enhanced pressure environment [8, 33].

Essentially, clinical hyperbaric medicine can be viewed as a relatively new application of an old established technology to help resolve selected medical problems [3]. Consequently, with this growing popularity, the number of hyperbaric units in the United States has grown from 34 to 260 facilities, from 1977-1998, with over 350 single-occupant (monoplace) chambers [36-38].

Currently, hyperbaric oxygen has several clinical indications for which it is the primary treatment modality: decompression sickness, air embolism and carbon monoxide poisoning [14,15]. Other conditions such as burns, crush injuries, compartment syndromes and osteomyelitis have also been shown to heal more rapidly with the application of hyperbaric oxygen therapy when used as an adjunct [14,16-20]. There has also been clinical investigation into the beneficial effects of hyperbaric oxygen and brain injuries [21,22]. Further research is underway examining its application for the treatment of multiple sclerosis [23-26] and its use is presently being investigated in children with cerebral palsy to determine whether it improves the quality of life for these children [27-29]. Recently, HBO has also been used in treating patients with HIV/AIDS, as it has been demonstrated to reduce the severity of, and secondary complications arising from, opportunistic infections [194].

Competitive sports have taken a whole new meaning in the last decade. Competition is fierce as athletes strive to be best in their field. However, this competitive nature in athletes has invoked a higher incidence of injuries in these players. These injuries, ranging from broken bones, torn muscles, tendons and ligaments, may be a result of acute impact forces in contact sports or the everyday rigors of training and conditioning [30]. This is where the field of sports and exercise medicine plays a crucial role in the rehabilitation of these athletes of all levels. Physicians are frequently challenged by athletes, coaches and trainers to provide new treatments to facilitate a speedier recovery [30]. One such treatment is the use of hyperbaric oxygen to accelerate the recovery process and allow the injured athlete to return to competition faster than the normal course of rehabilitation.

Table 1: Pressure Equivalents for Oxygen Compression

ATA (atmosphere absolute)	mmHg	FSW (feet of sea water)
1	760	0
2	1520	33
3	2280	66
6	4560	165

Table 2: Therapeutic Uses of Hyperbaric Oxygen*

Strong Scientific Evidence

Main treatment
Decompression sickness
Arterial gas embolism
Severe carbon monoxide poisoning and smoke inhalation

Adjunctive treatment
Prevention and treatment of osteoradionecrosis
Improved skin graft and flap healing
Clostridial myonecrosis

Suggestive Scientific Evidence

Adjunctive treatment
Refractive osteomyelitis
Radiation induced injury
Acute traumatic ischemic injury
Prolonged failure of wound healing
Exceptional anemia from blood loss

** adapted from Leach RM et al*[40]

Table 3: Select landmarks in the history and development of hyperbaric medicine, dating back to the 1600's

Date	Key Landmark
1662	J. Henshaw used compressed air for the treatment of a variety of diseases
1775	Discovery of oxygen by J. Priestley
1796	T. Beddoes and J. Watt wrote first book on medical application of oxygen
1837	C.G. Pravaz of France constructed largest hyperbaric chamber of that time to treat a variety of ailments
1860	First hyperbaric chamber on North American continent in Oshawa, Canada
1895	J.S. Haldane showed that a mouse placed in a jar containing oxygen at 2.0 ATA failed to develop CO poisoning
1921	O.J. Cunningham built a hyperbaric chamber in Lawrence, Kansas used to treat a variety of ailments
1928	O.J. Cunningham builds the largest chamber in the world in Cleveland
1937	A.R. Benke and L.A. Shaw first used HBO for treatment of decompression sickness
1956	I. Boerema, father of modern hyperbaric medicine, performed cardiac surgery in a hyperbaric chamber
1963	First International Congress on Hyperbaric Medicine in Amsterdam
1967	Undersea Medical Society founded in the USA. Now known as the Undersea and Hyperbaric Medical Society.
1970s	Extensive expansion of hyperbaric facilities in Japan and USSR
1983	Formation of the American College of Hyperbaric Medicine
1987	K.K. Jain demonstrated HBO integration with physical therapy
1988	Formation of the International Society of Hyperbaric Medicine

1.2 Combining Hyperbaric Oxygen with Delayed Onset Muscle Soreness: Putting the Pieces of the Puzzle Together

The delayed-onset muscle soreness (DOMS) model will elicit muscle injury and tissue inflammation in humans and serve as an excellent model to assess the efficacy of using this form of treatment in the rehabilitation of exercise-related injuries.

DOMS, characterized by high intensity eccentric contractions, clinically presents as increased stiffness, a decreased range of motion, tenderness, decline in force, swelling, electrically silent muscle shortening and a release of muscle enzymes in the blood [119, 182]. Rodenburg et al [224] has stated that "DOMS ultimately arises from a 'sequence of events' occurring after eccentric exercise, including myofibrillar disruption, increased permeability of the sarcolemma to muscle proteins, free radical release and inflammatory processes, with the latter possibly leading to DOMS. This 'sequence of events' is initiated by mechanical stress on the muscle fibres, metabolic overload or a combination of both".

Due to the lack of scientific evidence supporting the efficacy of using hyperbaric oxygen therapy in sport and exercise-related injuries, the effects of using this treatment modality was looked at in an acute soft tissue inflammatory condition, namely DOMS. This premise is based on the mechanism of action associated with HBO, and the mechanical and biochemical processes that occur during a bout of DOMS. Muscle damage, muscle soreness and loss of muscle function will be determined by assessing isokinetic strength decrements, associated perceived muscle soreness, elevation in blood enzyme levels of creatine kinase, interleukin 6 and malondialdehyde, as well as magnetic resonance imaging of edematous tissue.

Each piece of the "puzzle" can be put together to determine whether, in fact, hyperbaric oxygen does have an important role in the future of sports and

exercise medicine. If it does, further research can then be conducted on a wide array of other injuries acquired in competitive and recreational activities as well as other clinical disorders

1.3 Hypothesis

The purpose of this study was to determine whether intermittent exposure to hyperbaric oxygen in the treatment group, compared to the control group, would increase the rate of recovery from DOMS, thus:

1. Reducing perceived muscle soreness over the five-day testing period.
2. Significantly improving eccentric muscle strength during the recovery of DOMS.
3. Decreasing elevated creatine kinase (CK) levels associated with skeletal muscle damage over the five days, thereby bringing CK levels close to pre-exercise levels by day 5.
4. Decreasing elevated interleukin-6 (IL-6) levels associated with exercise-induced muscle injury over the five days, thereby bringing IL-6 levels close to baseline by day 5.
5. Decreasing elevated malondialdehyde (MDA) levels associated with lipid peroxidation as a result of muscle injury over the five days, thereby bringing MDA levels close to pre-exercise levels by day 5.
6. Reducing edema in the quadricep muscle of the non-dominant leg over the five-day testing period, as evidenced by magnetic resonance imaging (MRI).

1.4 Assumptions

The following assumptions were made when designing the study:

1. All subjects will respond honestly, to the best of their knowledge, regarding the amount of activity that they perform on a weekly basis as well as their involvement in competitive sporting activities.
2. All subjects will report accurate pain scores, to the best of their ability, when filling out the visual analog scale.

3. Performing 300 eccentric contractions will create muscle injury and tissue inflammation (DOMS) in the quadricep muscle of the non-dominant leg.

1.5 Limitations

The study, at the present time, was limited by the following:

1. The severity of soreness, as perceived by individual subjects, may be prone to inter-subject variability.
2. Subject recruitment: It was extremely difficult to recruit subjects for the study due to the time commitment required over the 5 days of treatment and the travel distances required by the subjects (Allan McGavin Sports Medicine Clinic, Buchanan Exercise Science Laboratory, UBC, St. Paul's Hospital and MRI Vancouver).

1.6 Delimitations

1. Sample selection; sedentary or relatively sedentary individuals, between the ages of 18-40 years (N=16; n=8 per group), were recruited to maximize the extent of DOMS since their quadricep muscle will not be adapted to eccentric loading.
2. The Kinetic Communicator (KinCom) Dynamometer was used to test the subject's muscle strength through a specific range of motion while performing isolated knee extensions.
3. The DOMS protocol (eccentric exercise protocol) allowed the investigator to control the insult of the injury.
4. The circumference measurement of the quadricep muscle evaluated the girth of the thigh, including skin, fat, muscle, bone and possible edema occurring within the musculature.
5. IL-6 measurements taken at baseline and repeated throughout the five-day testing period demonstrated the cytokine response during inflammation and DOMS.

6. Malondialdehyde measurements taken at baseline and repeated throughout recovery were indicative of lipid peroxidation during muscle injury.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Effects of Hyperbaric Oxygen in Wound Healing and Tissue Survival

During a soft tissue injury, a disruption of cells and blood vessels occur within the tissue, resulting in hypoxia. This is followed by a subsequent aggregation of platelets and collagen to the area of injury. In addition, an increase in extracellular fluid and vascular dilation occurs, followed by an influx of neutrophils, macrophages, fibroblasts, smooth muscle cells and endothelial cells to cleanse and reconstruct the insult. Lactate, hypoxia and the production of cytokines further causes growth stimulation, which leads to angiogenesis and the production of collagen. The wound is essentially considered healed after the occurrence of this cascade of events [41-42, 50].

Tissue damage can lead to edema, complications with blood flow and eventual tissue death, including ischemia [8, 41-42]. The increase in extracellular fluid and vascular dilation impairs oxygen delivery from capillaries to cells because of an increase in the diffusion distance [9]. Resulting decreases in oxygen tensions (e.g. below 30 mmHg) make the cells more susceptible to infection [7, 43] and the efficacy of leukocytes in reducing invading organisms becomes defective [12, 44]. Furthermore, host repair processes are also impaired during this time [7].

Oxygen, therefore, plays a critical role in the wound healing process. It serves as a catalyst and energy source for maintenance, metabolism and repair [45, 46]. In wound healing, oxygen serves to provide the additional energy source necessary for the reparative process. Early in the repair of wounds, fibroblasts begin to migrate, divide and produce collagen, which is an essential matrix for wound healing. Oxygen must also be present in sufficient quantities for fibroblast proliferation and collagen production to occur. Adequate amounts of proline and lysine (two amino acids incorporated by oxygen) must be hydroxylated with oxygen for collagen to be synthesized by fibroblasts [47]. Oxygen must also be present in adequate amounts during the repair process to provide energy for protein synthesis. It has been demonstrated that raising oxygen tension in

tissues increases the ratio of RNA/DNA [48]. Pal et al [49] have stated that an increase of 150% above the normal physiologic range for oxygen (PO_2=40 mmHg) increases the rate of collagen production by seven times.

An adequate delivery of oxygen via an extensive capillary network is essential since the diffusion of oxygen through tissues is limited [46]. Disruption of the capillaries from trauma produces hypoxia and the release of hormonal mediators. Macrophages release an angiogenesis factor, which is a potent stimulus for endothelial cell activity [50]. Polymorphonuclear cells (PMN) which locate, identify, phagocytose, kill and digest microorganisms, require oxygen to kill organisms by producing superoxide, hydrogen peroxide, singlet oxygen and other products via the respiratory burst [51]. Detoxifying free radicals by superoxide dismutase, catalase and glutathione protect the PMNs. Therefore, the degree of PMN cell function in killing of bacteria is directly dependent on oxygen tensions [44, 52].

Hyperbaric Oxygen has two components in wound healing and tissue survival: acting *mechanically,* due to its pressure component and *physiologically,* due to its oxygen component [39]. The oxygen content is determined by a combination of oxygen that is bound to hemoglobin plus the amount of oxygen that is dissolved in the plasma.

[Total O_2 = hemoglobin bound oxygen + oxygen dissolved in plasma]

The application of hyperbaric oxygen increases the amount of available oxygen to the hypoxic area of injury, increasing oxygen tensions that make host repair processes functional [8, 9]. Increased availability causes a shift in the oxygen cascade (a gradient from the partial pressure of oxygen in the ambient air to that available immediately to the tissues on a cellular level). This effect of hyperoxygenation is based on a combination of two laws, namely *Henry* and *Dalton's* Laws. Henry's Law states that as the PO_2 increases during compression,

the amount of oxygen dissolved directly into the plasma increases [3, 53]. By increasing the partial pressure of oxygen in the air, a significant amount of oxygen becomes dissolved in the blood plasma. Furthermore, Dalton's Law states that air is a mixture of gases and that the total pressure exerted is the sum of the partial pressures of each of the gases in the mixture [53]. These two laws combined produce the effect of hyperoxemia, which allows enough oxygen to dissolve in the plasma to meet metabolic needs. At sea level in room air, there is 0.32 ml of oxygen dissolved in each 100 ml of whole blood (0.32 vol.%). When breathing 100% oxygen, each additional atmosphere of pressure produces an additional 2.3 vol.% oxygen dissolved in plasma. At 2.0 ATA, the blood oxygen content increases 2.3% while plasma and tissue oxygen tensions increase tenfold (1000%) [8,9,11,22,54]. Consequently, sufficient oxygen becomes physically dissolved in the plasma to keep tissues alive despite the inability of hemoglobin-bound oxygen to reach the insulted area [17, 55]. At 3.0 ATA, the partial pressure of oxygen in the blood can increase to as much as 2200 mmHg (plasma contains 6.8 vol.% oxygen). This elevated oxygen pressure increases the oxygen diffusion gradient and improves oxygen delivery to relatively ischemic tissues [53-56]. At this pressure, enough oxygen can be dissolved in the plasma to sustain life temporarily without any red blood cells [57].

Secondary effects of hyperbaric oxygen therapy include vasoconstriction, gas volume reduction, inhibition of anaerobic organisms and neovascularization. Vasoconstriction causes a decrease in edema at the wound site leading to decreased tissue perfusion without sacrificing oxygenation [53]. Sufficient amounts of oxygen are dissolved in the blood plasma to adequately compensate for the decrease in blood flow to the area of injury [9, 58]. This reduction in blood flow produces a subsequent 20% reduction in post-traumatic vasogenic edema [59]. Hyperbaric oxygen therapy also causes a reduction in gas volume by reducing the size of the gas bubbles. This physiological effect is based on Boyle's law stating, "the volume of a gas is inversely proportional to its pressure at a constant temperature". Therefore, as the pressure increases, the volume of

a gas decreases, reducing the size of gas bubbles that impede circulation [53]. Because of this effect, HBO is used as the primary treatment in air embolisms and decompression sickness [60].

Although aerobic organisms continue to thrive when given increased amounts of oxygen, their growth may be inhibited when the partial pressure of oxygen exceeds 1.3 ATA [57, 61]. However, inhibition of aerobic organisms is best achieved when they are superficial [62] (e.g. a superficial ulcer or burn). Hyperbaric oxygen therapy used as an adjunct has also been demonstrated to be beneficial in treating anaerobic infections such as gas gangrene and inhibition of alpha toxin production [62] [Table 4].

Table 4: Cellular and Biochemical Benefits of Hyperbaric Oxygen*

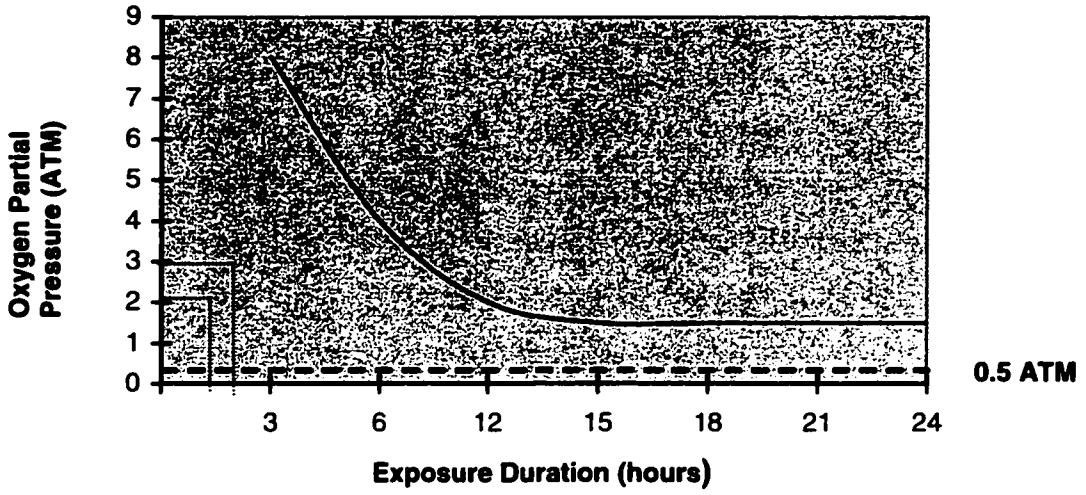
- Promotes angiogenesis and wound healing
- Kills certain anaerobes
- Prevents growth of species such as Pseudomonas
- Prevents production of clostridial alpha toxin
- Restores neutrophil mediated bacterial killing in previously hypoxic tissues
- Reduces leukocyte adhesion in reperfusion injury, preventing release of proteases and free radicals which cause vasoconstriction and cellular damage

**adapted from Leach RM* [40]

2.2 Oxygen Toxicity Related to Hyperbaric Oxygen Therapy

Oxygen in large amounts, like most therapeutic modalities, can be toxic and life threatening. However, it has widely been reported that toxicity is related to both the duration of exposure and pressure [63] (Figure 1). Oxygen toxicity affects both central and pulmonary nervous systems. Oxygen toxicity involving the central nervous system is termed the *Paul Bet effect* and results in seizures [205]. Oxygen toxicity affecting the pulmonary system is termed the *Lorrain-Smith effect*, and causes edema in the lungs and subsequent alveolar collapse [206].

Figure 1: Pressure-duration relationship for effects of oxygen toxicity when using hyperbaric oxygen therapy [63]



Central nervous system associated grand mal seizures have been documented at a pressure of 3.0 ATA, [62, 63] while pulmonary edema has been detected at 2.0 ATA [54, 64-66]. However, both toxic effects occurred at prolonged exposures of three or more hours [9, 65, 66, 68]. Davis [67] estimated the incidence of oxygen seizures to be one in 11,000 treatments. Thom [207] reported that the likelihood of central nervous system agitation is approximately 0.0009%. Factors contributing to a seizure are fever, exercise, apprehension and CO_2 buildup [57]. Typical warning signs may include tunnel vision, shortness of breath, tinnitus, nausea and extreme apprehension. If a seizure occurs during treatment, the patient should be removed from the chamber but not until the seizure has stopped. To decompress the patient during the tonic phase of the seizure can put the patient at risk of air or oxygen embolism [57]. Seizures, if promptly treated, have no permanent sequelae.

Pneumothorax can occur under hyperbaric conditions due to predisposing lung pathology such as gas trapping in a localized portion of the lung [57]. The patient may experience respiratory distress, sudden stabbing chest pain, a shift of the trachea toward the unaffected side, lack of chest movement on the affected side, decreased breath sounds on the affected side and increased tympany [57]. When a multiplace chamber is used, treatment of pneumothorax can be

managed by inserting a needle or chest tube into the affected side. For treatment in a monoplace chamber, the patient must be decompressed before treatment. This may cause the pneumothorax to double or triple in volume but respiratory distress may subside when recompression occurs.

Pulmonary oxygen toxicity may be a consideration in patients maintained on an inspired oxygen fraction of greater than 40% between hyperbaric oxygen sessions [208]. In this instance, the clinician on hand must be aware of the hyperbaric oxygen treatment to monitor for toxicity and intervene if the need arises.

The uses of aspirin, insulin, steroids, epinephrine and norepinephrine have all been noted to increase the onset of oxygen toxicity [9, 34]. Concurrent therapy with doxorubicin (Adriamycin^{R}), cis-platinum and disulfiram (Antabuse^{R}) are also incompatible with HBO [3]. When combined with HBO therapy, doxorubicin has produced a high mortality rate in animals. Furthermore cis-platinum used concurrently with HBO therapy decreases the strength of healing wounds and disulfiram blocks production of superoxide dismutase, the body's major protection against oxygen toxicity [3]. Concerns have also been expressed as to whether physical exercise predisposes patients to oxygen toxicity [9] but a study conducted by Stevens et al [66] concluded that there were no adverse effects of oxygen toxicity with exercise. Conversely, antioxidants such as vitamin C, vitamin E and mexamine, lithium, and magnesium have been used to prevent or delay the onset of toxicity [202-204]. The best way, however, to prevent prolonged oxygen toxicity is to take periodic breaks, inspiring normal air (21% oxygen) [9]. A five year prospective study exposing 12 468 patients to 100% oxygen at 2.0, 2.2 and 2.4 ATA showed no oxygen-related complications or seizures at 2.0 ATA [68]. Mild aural barotrauma was the only complication observed in this study. Barotrauma is defined as any injury to structures such as the ear due to differences between atmospheric and intratympanic pressures (commonly referred to as "squeeze") [57]. Due to pressure changes associated

with hyperbaric treatment, the patient must be taught to equalize the pressure in the ear by swallowing, yawning or using the valsalva manoeuver. The primary symptom of "squeeze" is pain in the ear while the chamber is being compressed [57]. Barotrauma can also occur in the sinus cavity, primarily the frontal sinus. Decongestants are usually prescribed when this occurs. In a series of studies conducted, Davies reported that one in 270 cases had barotrauma significant enough to interrupt treatment [67]. Others have stated complications such as nausea, tooth and sinus pain and blurred vision [9]. Therefore, it can be concluded that no toxic effects are seen within the current therapeutic range of 2.0 ATA at a 60-90 minute exposure time, accompanied by frequent air breaks during treatment.

Proper screening is essential before hyperbaric treatment can be administered. Contraindications for HBO therapy include upper respiratory tract infections, diabetes, pregnancy, confinement syndrome, pneumothorax, sinusitis and fever [Table 5].

Fire in the 100% O_2 environment of a mono/multiplace chamber is, although rare, a concern that should be addressed. All efforts should be maintained to minimize the risk, including the exclusion of items that could be associated with heat or flame. These include velcro, glycerin (including hair products and cosmetics), perfumes, lotions and non-cotton fabrics (should be 100% cotton to reduce static) [Table 6].

Oxygen toxicity is a serious consequence of treatment with hyperbaric oxygen. However, this can be controlled with proper screening of the patient prior to administration of treatment and appropriate control over length of exposure and pressure levels. Ideally, it can be concluded that treatments for 60-90 minute durations are within a reasonable level to avoid complications that may arise. Furthermore, treatment should be administered at 2.0-2.5 ATA, accompanied by frequent air breaks.

Table 5: Contraindications to HBO Therapy*

- Pneumothorax
- Uncontrolled high fevers
- Severe chronic obstructive pulmonary disease
- Optic neuritis
- Acute viral infection
- Congenital spherocytosis
- Upper respiratory tract infections
- Pregnancy
- Psychiatric problems
- Prior thoracic or ear surgery
- Acute seizure disorders

*adapted from Foster J [69]

Table 6: Patient Preparation Prior to HBO for Safety and Fire Prevention

- The patient should shower or bathe daily and hair should be shampooed if oily before HBO
- Body oils, lotions, talc, petroleum products, make-up, cologne, perfume, deodorants, nail polish, hair oils and spray are not to be used before treatment.
- Only all cotton clothing and linens are allowed in the chamber to prevent development of static electricity.
- Wigs or hairpieces, hearing aids, jewelry and contact lenses are not to be worn in the chamber.

*adapted from Curtis et al [53]

2.3 Previous Studies Examining HBO and Tissue Injuries

Burns

A vast amount of research exists on the effects of HBO on burns, especially thermal burns. First documented in the 1970's, patients exposed to HBO found that burns dried sooner, had fewer infections and healed more quickly [18, 70]. Healing time was shown to decrease by 30% in more recent controlled studies [10, 71-73]. Other authors examining second degree burns report increased rates of epithelialization, decreased fluid requirements, preservation of microcirculation, decreased conversions from partial to full thickness injury, reduction in edema and inflammatory response and decreases in grafting and surgical procedures, hospital time and relevant associated costs [3, 5, 72-74]. In a model scald burn, Nylander et al [76] demonstrated a decrease in global edema following an ear burn. Stewart et al [75] examined ATP, phosphocreatine and collagen synthesis in burn wounds of rats. They concluded that as an adjunct, HBO could preserve or enhance energy rich phosphate compounds and collagen synthesis in model burn wounds.

Although large amounts of research exist demonstrating the healing potential and beneficial effects on epithelialization, some discrepancy does exist in the literature with respect to the efficacy of HBO therapy as an adjunct treatment. Other controlled, randomized human studies show no effect in the healing process [43, 76]. This discrepancy may be attributable to the time lag from injury to the initiation of treatment, possibly 24-48 hours post-injury.

Infections

The role of oxygen in fighting infections has also been established. Oxygen acts as a potent antibiotic, improving the ability of special scavenger white blood cells (phagocytes) to rid the body of bacteria and other foreign proteins. Hyperbaric oxygen has a distinct antimicrobial effect that is equal to or better than that of numerous antibiotics [29]. This form of treatment may also enhance the actions of certain antibiotics and thereby increase their effectiveness in overcoming

infections [29]. Because of its immune system-enhancing effects, hyperbaric oxygen therapy helps fight all microorganisms, both anaerobic and aerobic organisms. The literature has reported over 4,000 cases of gas gangrene treated with hyperbaric oxygen therapy. One study reported a survival rate of 88.3% among 248 patients who received treatment [209]. Another study of 139 patients reported a survival rate of 70 percent, with 80% of the survivors able to avoid amputations [210]. Ellis and Mandal analyzed 58 patients who failed to respond to conventional treatment of antibiotics and surgery. After treatment with hyperbaric oxygen, there was a survival rate of 84% [211].

Wound Healing

A wound is any disruption in the body's tissues. It is often associated with the loss of skin (and underlying tissue), muscle or bone. Many wounds respond to conventional medicine while others do not. Primarily, a wound lacks the necessary oxygen required for healing to take place. This may be due to a blood clot, which interferes with the circulation. As a result, the oxygen supply to the area of insult is diminished and toxic materials accumulate since waste products cannot be removed. This starts a cycle of damage to affected tissues. Wounds are also prone to infection since the lack of oxygen in the tissues can reduce the injured person's defense by decreasing the activity of infection-fighting white blood cells [12]. Underoxygenation can also deactivate the cells that produce granulation tissue and interfere with collagen production [194]. Therefore, it can be concluded that a lack of oxygen in the wounded tissue can interfere with the entire wound healing process.

In the treatment of open wounds, HBO plays a vital role in collagen synthesis, hydroxylation of the collagen molecule and intracellular ATP production, thus promoting tissue repair and growth [7, 8]. To summarize, HBO promotes granulation tissue formation, revascularization, epithelialization, enhanced fibroblastic activity and leukocyte killing, fibroblast migration, angiogenesis as well as capillary budding [3, 36, 78-80]. Ample clinical investigations have

demonstrated that in hypoxic wounds (PO_2 = 5-20 mmHg), tissue healing occurs with sufficient tissue oxygenation, which is enhanced by HBO [81]. In a study of patients with chronic diabetic foot lesions, Doctor et al [184] observed a better control of infection and less need for amputation in the group treated with conventional management and sessions of HBO.

Bone Healing and Hyperbaric Oxygen Therapy

Bone is composed of three layers: a spongy inner layer, a rigid middle layer and a tough outer layer. When bone is injured, special cells called osteoclasts work to repair the damage. These cells carve paths through the bone tissue around the brake and cause dead bone to be reabsorbed by the body. Osteoblasts, another group of cells, then create new bone [29]. These osteoclasts depend and utilize oxygen for proper function. Therefore, hyperbaric oxygen may facilitate bone healing by stimulating both osteoclasts and osteoblasts [7, 8]. It may also stimulate the production of new blood vessels, so that the growing bone receives a steady supply of nutrients, including oxygen. This blood-vessel network helps support the function of the osteoclasts and brings infection-fighting white blood cells to the area of injury [29].

Osteomyelitis

Osteomyelitis is a bacterial infection that usually occurs on the outer layers of bone as well as the inner bone marrow. Staphylococci bacteria are primarily implicated in this type of infection. The germs that cause this infection can enter the bone during an injury or surgery. Furthermore, it may also reach the bone directly from a nearby infection or indirectly through the bloodstream. Osteomyelitis may be either acute or chronic, with severe pain, swelling and redness at the site of infection in the acute phase of infection. High fever is also prominent in patients with osteomyelitis. In the chronic stage, symptoms include bone pain, tenderness and local muscle spasm.

Conventional treatment for osteomyelitis includes antibiotics and several weeks of bed rest. Surgery may be required to take out dead bone and soft tissue, fill holes and implant artificial devices designed to keep the diseased bones and joints from moving [29]. It is imperative to treat the bone infection promptly and vigorously in order to prevent it from spreading to other parts of the body.

Hyperbaric oxygen has three main functions in treating osteomyelitis. First it helps strengthen the bone cells (osteoclasts) that reabsorb dead bone, removing bony debris more effectively. Second, it enhances the function of the immune system's white blood cells, since they depend on oxygen. This is especially effective when used with antibiotics [29]. Lastly, hyperbaric oxygen helps the body to create new blood vessels. Through these mechanisms, hyperbaric oxygen enables the body to get rid of the diseased bone and replace it with healthy bone [29].

Animal research on the effectiveness of hyperbaric oxygen for the treatment of osteomyelitis dates back to the late 60's. These investigators demonstrated that animals were cured of bone infections with the use of hyperbaric oxygen alone (i.e. no antibiotics or surgery). Furthermore, they treated another group of rats prior to inducing infection but found that it did not prevent the infection from taking hold. The authors concluded that hyperbaric oxygen was an effective treatment modality in treating osteomyelitis because it enhances the host's own immune system and not because it kills the bacteria directly [212].

The benefits of hyperbaric oxygen have also been established in human treatments. Neubauer et al [29] reported that the overall success rate in various investigations using hyperbaric oxygen therapy on osteomyelitis ranges from 60 to 85%, with a lower rate of recurrence. Davis [213] demonstrated in a five year follow-up study, that in 136 patients with refractory chronic osteomyelitis (cases which failed to heal with antibiotic treatment or surgery) of the spine, extremities, pelvis, skull and chest wall, over half of these patients had their infection clear up.

Another study demonstrated a cure rate of 85% in a two-year follow-up study of 40 patients with chronic osteomyelitis. Hyperbaric oxygen in this study was used as an adjunct to surgery and antibiotics [214].

Aseptic Bone Necrosis

Aseptic bone necrosis (ABN) occurs when bone becomes inflamed without being infected. It usually occurs as a complication of decompression sickness but can occur in diabetes, hepatitis, rheumatoid arthritis and sickle cell anemia. It may also result as a side effect of various therapeutic procedures such as radiation therapy and steroid treatment [29]. ABN can also arise spontaneously in the general population, especially in children ages eight to fourteen [215-216].

ABN is essentially a blood supply problem [217]. This blood vessel disruption results in ischemia or a reduced blood supply resulting in bone not getting enough nutrients and oxygen. In addition, cellular wastes accumulate [217].

Aseptic bone necrosis should be treated properly as multiple joints are often involved and may lead to permanent disability. Widespread ABN requires a great deal of joint-replacement surgery, causing a large amount of expense, pain and disability [29].

This is where hyperbaric oxygen therapy fits in. It seems logical that increasing the amount of oxygen in the affected tissues may halt deterioration and promote healing. Neubauer and colleagues [29] have concluded that hyperbaric oxygen does indeed help patients with ABN, however treatment should be long-term. Short-term treatment does relieve pain and disability but no lasting cure takes place [218-219].

Fractures

Clinical investigation on the use of HBO on fractures has been promising, especially where non-union and complicated fractures with increased chance of

infection are involved [82]. Findings such as increases in osteoblastic DNA and RNA, bone mineralization, hematoma and callus formation, callus nitrogen content, capacity for protein synthesis, alteration of the homeostatic environment, reduction in osteoblast formation, collagen formation, capillary budding, fibroblastic activity and proliferation and osteogenesis have all led to the conclusion that HBO speeds recovery in fractures and reduces healing time [75, 83, 85]. Researchers have concluded that hyperbaric oxygen therapy leads to greater cartilage production and bone formation [220]. It has also been demonstrated to help in bone grafts [221]. In an animal study of 487 fractures in and around the joints, researchers concluded that hyperbaric oxygen therapy used as an adjunct to conventional orthopedic methods, shortened the process of bone regeneration and wound healing by ten to twelve days [222].

Again, contradiction does exist in the literature whereby other authors observe no decreases in healing time [86, 87]. This may be due to the time of onset of treatment from the actual injury time (12 hours or more after the injury).

Crush Injuries / Traumas / Compartment Syndrome

Crush injuries involve diffuse blood loss resulting in tissue hypoxia, which severely affects cellular function [19]. In addition, increased blood flow resulting in edema accompanies the insult. This edema causes an increased diffusion distance for oxygen to travel to the capillaries [9]. In closed crush injuries, all circulation to the injured area is diminished [88]. HBO serves to cause a 20% reduction in blood flow in addition to providing a sufficient medium for macrophage to function [44, 45]. Clinical trials have demonstrated beneficial effects such as 1) countering wound infection accompanying open traumas of the extremities 2) accelerating the recovery of neutrophil phagocytic activity 3) prevention of limb amputation and 4) healing the open fracture without suppuration (dead skin with a distinct line of demarcation) [13, 66]. However, the above studies failed to compare the HBO treated patients with those treated more conservatively, thus not illustrating a clear difference in healing patterns. In

a study demonstrating the effects of HBO on the management of severe trauma of the limbs in older patients with grade III soft-tissue injuries, Bouchard et al [89] found HBO to improve wound healing and reduce the repetitive surgery necessary in cases of aggravation of crushing tissue damage. Nylander et al [90] exposed rat hind limbs to 3 hours of temporary ischemia followed by 45 minutes of HBO sessions at 2.5 ATA. The results showed a significant reduction in post-ischemic edema and thus concluded that HBO was a useful adjunct in the treatment of acute ischemic conditions when surgery couldn't be attempted or failed to reverse ischemia [76, 90, 91]. Jones et al [92] conducted a preliminary study on the effects of HBO on ten patients with compressive lesions of the spinal cord leading to paralysis. The results suggested that by supporting injured spinal cord tissue with oxygen under pressure, improvement might occur. A large series of patient, in this type of study, will be necessary before definite conclusions can be drawn on whether HBO therapy improves recovery in the paralyzed patient with a bruised spinal cord. Studies conducted on animal models for compartment syndrome (increased pressure in skeletal muscle compartments causing reduced capillary perfusion, leading to ischemia, nonfunctional and necrosis of tissue) have all showed promising results of reduced muscular necrosis and edema in HBO treated versus control groups [81, 84]. Zamboni et al [93,94] have also conducted several experiments examining the effects of hyperbaric oxygen on ischemic muscle on animal models, again with positive results showing a reduction in edema and improved microvascular perfusion. Further research, however, needs to supplement the existing body of literature in terms of human, controlled, double-blinded studies.

Hyperbaric oxygen therapy can provide various benefits in the treatment of wounds. To summarize: it encourages the growth of new tissue by providing extra oxygen, it counteracts the chance of infection by indirectly providing cells (i.e. white blood cells) the extra oxygen that they need and directly by killing anaerobic organisms, stopping their multiplication and neutralizing the toxins that some produce [12], it encourages bone repair by supplying osteoclasts and

osteoblasts the rich supply of oxygen that they require and lastly it provides a clear line of demarcation between tissue which is beyond repair and that which can be saved [223]. Therefore, the effectiveness of hyperbaric oxygen therapy has been beneficial in minimizing tissue death, reduction in swelling and promoting healing.

2.4 The Role of Hyperbaric Oxygen in Sports and Exercise Medicine

There is a considerable amount of significant research suggesting the efficacy of HBO therapy as both a primary and adjunct in the treatment of a variety of illnesses and injuries. However, only a paucity of knowledge exists in terms of its use, benefits and mechanism of action in sport and exercise-related injuries [Table 7,8]. Although the few studies conducted thus far looking at acute injuries in sports and exercise have proven to be promising in terms of using HBO as a treatment modality, these studies have been limited by their sample size and study design [1, 2]. Leach et al states, "*the gap between the knowledge gleaned from the laboratory and severely traumatized patients and the athlete in the locker room remains vast* [40]". This statement clearly summarizes how much research needs to be conducted to establish or refute the role of hyperbaric oxygen therapy in sports and exercise medicine.

> Hyperbaric Oxygen - "Applications of HBO arise as a result of medical adventurism; A therapy in search of a disease"
>
> *Gabb G & Robin ED* [70]

Studies to date

Oriani et al [88] first suggested the use of HBO to accelerate the rate of recovery from injuries suffered in sports. However, it wasn't until recently that a study on HBO was first published. This study looked at the number of days lost to injury in professional soccer players in Scotland [1]. The results suggested a 55% reduction in days-lost-to-injury based on a physiotherapists estimation of the time-course for the injury versus the actual number of days lost with routine therapy and HBO treatment sessions. Although promising, this study was subjective in nature, needed a control group, input from an objective third party

and required a greater homogeneity of injuries. Randomized, controlled, double-blinded studies with quantifiable injuries are required for significant validity of results.

An ankle inversion study conducted at Temple University suggested that patients exposed to HBO treatments returned to activity 30% faster than control groups [2]. Unfortunately, the results were inconclusive in this study since a large amount of variability existed in the study design. The authors attributed much of this observed variability to the difficulty in quantifying the severity of ankle sprains [33]. Furthermore, this study has been countered by a recent randomized double-blind design in which HBO treatment did not improve time to recovery after an acute ankle sprain injury [95].

Staples et al [96, 97] conducted both an animal and human study on a muscle injury model. The animal model, which measured myeloperoxidase levels in treated versus untreated rats, was suggestive of an inhibitory effect of hyperbaric oxygen on the inflammatory process or the ability of HBO to actually modulate the injury to the tissue. The human study employed a randomized, double-blinded design with controlled start and end points. The promising results revealed that the treatment group with HBO had a greater recovery of eccentric strength from delayed-onset muscle soreness (DOMS). However, this treatment modality had no effect on pain levels [96, 97].

One of the best quantitative studies to date comes from a rat model of surgically lacerated medial collateral ligaments. This study compared ligament strength and stiffness in injured and uninjured ligaments over an 8-week period and concluded that the HBO appears to have promoted the return to normal stiffness of the ligaments at 4 weeks as well as enhanced recovery of ligament strength [98].

Another clinical study completed by Soolsma et al [32] at the University of British Columbia examined the short-term recovery of grade II medial collateral ligament injuries of the knee. Positive results suggest that recovery was more rapid in the HBO exposed individuals as compared to the control group [32].

A recent study completed by Best et al [99], demonstrated in a rabbit model, that a 5-day treatment regimen with HBO appears to improve functional and morphologic recovery at 7 days after a controlled reproducible muscle stretch injury.

Countering the above studies is the work conducted by Harrison BC et al, at the University of Colorado, which examined the effects of treating exercise-induced muscle injury via hyperbaric oxygen. These researchers concluded that HBO was not effective in the treatment of muscle injury as evidenced by MRI, CK response, isometric strength testing and perceived soreness [246].

Mekjavic et al, have also recently reported that HBO is not effective therapy for the treatment of DOMS based on maximal isometric muscle strength of the elbow flexor muscles, right upper arm circumference and ratings of perceived muscle soreness [225].

Although few in number, all of the above studies provide some insight into the application of HBO as an adjunctive treatment in sports and exercise medicine, and warrants the need for additional research to better define the therapeutic indications of hyperbaric oxygen.

The demand for hyperbaric oxygen therapy is increasing throughout the medical field. Medical professionals, clinicians and researchers have been and are currently venturing on new areas that haven't previously been investigated. A growing interest in sports and exercise medicine is appearing throughout the literature. However, with this new spark of enthusiasm comes a high degree of

skepticism that has been developed regarding its use. To date only a handful of studies exist in this area and of these studies, only a select few support the benefits of utilizing this intervention.

Table 7: Benefits of HBO in Sports Injuries*

- Reduction of pain and swelling in the acute stage
- Speeds up recovery and return to activity training
- Improves fracture healing
- Aids in the recovery from exhaustion and collapse

* *Jain KK* [9]

Table 8: Proposed Mechanism of Action in Sports Injuries*

- Vasoconstriction
- Reduction in neutrophil-adhesion
- Free radical quenching ability
- Enhancement of leukocyte killing and hydroxyproline formation

* *Jain KK* [9]

2.5 Acute Soft Tissue Injury: Delayed Onset Muscle Soreness

Characteristics of DOMS

At some point in time, nearly all of us have experienced the sensation of delayed-onset muscle soreness (DOMS); many of which have had numerous encounters with this common self-limiting ailment. Even trained individuals will experience some soreness following a novel bout of unaccustomed exercise [100]. This condition is usually characterized by a sensation of pain and discomfort that occurs in skeletal muscles following a bout of unaccustomed exercise and exertion, and is often accompanied by tenderness and stiffness, with a reduction in mobility or flexibility of the muscles involved. This reduced mobility and flexibility is exacerbated during palpation, passive stretching and contraction of the involved muscles [101-103]. The pain may be slight and may disappear upon repeated activity or may be severe enough to interfere with and limit

movement. This pain and tenderness is usually localized to the distal third portion of the muscle, in the region of the muscular-tendinous junction where muscle pain receptors are most concentrated, with eventual spreading to the center of the muscle belly by 48 hours [104]. Newham et al [104] have reported that the pain associated with DOMS appears medially, laterally and then distally, becoming more diffuse throughout the muscle 24-48 hours post-exercise. Generally, however, the pain is evident throughout most of the affected muscle belly. The soreness that is prominent with DOMS initially appears 8-24 hours after exercise, increases in intensity within the first 24 hours, peaks from 24-72 hours and finally subsides 5-7 days post-exercise [102, 105, 106].

Type of Activity Inducing Muscle Injury

High intensity, short duration exercise results in the largest increase in muscle damage and DOMS [107]. Eccentric exercise (e.g. downhill running - involving forced lengthening of a muscle as it develops tension [108]) has been shown to produce greater damage to the muscle fibres as opposed to concentric contractions (e.g. uphill running), since the former requires lower energy costs as fewer motor units are activated for a given load [104, 109, 110]. At a given submaximal force or power output, EMG activity in muscle has been demonstrated to be lower during eccentric (negative) work than concentric [111, 112]. This leads to higher tensions per cross-sectional area of active skeletal muscle fibres [104, 110]. The increased tension could cause mechanical disruption of the structural elements in the muscle fibres themselves [102, 104, 110, 113] or in the connective tissue that is in series with the contractile elements [114]. Evans [115, 116] suggested that the reason why eccentric exercise causes far greater amounts of muscle damage than concentric exercise might be due to different fiber recruitment patterns. In other words, eccentric contraction conditions provide a situation where relatively few fibres are recruited and are producing relatively large forces [110]. Cleak [105] reported that lengthening muscle fibres imposes additional tension and stretch on connective tissue and appears to increase localized soreness in the region of the muscle involved.

Cannon et al [108] cite that repeated force lengthening of a muscle as it develops tension causes immediate ultrastructural damage to the sarcomeres, followed by delayed-onset muscle soreness and a release of myocellular enzymes. Consequently, it has been reported that eccentric work requires less oxygen, lower amounts of ATP, produces less lactate and shows lower motor unit activity than concentric work [114, 117-120]. Furthermore, eccentric contractions require less time to reach peak tension [113].

More recent evidence suggests that muscle damage is not a function of absolute force or tension generated but the magnitude of strain during active lengthening [121]. These authors determined that active strain during eccentric work was related to the speed at which a muscle was lengthened while it was contracting. It has been suggested that the greater damage elicited by faster eccentric contractions was due to the fact that cross-bridge cycling could not keep pace with the change in length of the muscle [122].

Muscle fiber type has also been reported to be a cause for muscle damage. It has been suggested that specific damage can be noticed in the type 2B fast glycolytic fibers, thus hypothesizing that muscle injury may be a result of the muscle fiber oxidative capacity [227]. It is believed that type 2 fibers might be more susceptible to stretch induced injury because of a less developed endomysium that type 1 fibers [228].

From a review of the literature, it seems apparent that muscle damage may not only be a result of high force generation but the magnitude of strain during active lengthening may also play a role in the injury process.

Eccentric Strength Loss Accompanying DOMS

Throughout the literature, it is evident that DOMS is a consequence of muscle "over-use" [100-107]. Any activity in which the muscle produces high forces or forces over a longer period of time is capable of producing the sensation of

DOMS [107]. In addition, although the degree of soreness is related to both the intensity of the muscular contractions and the duration of exercise, intensity seems to be the more important determinant of the two [101, 107, 123]. Hough [124] was the first to describe in detail the phenomenon of DOMS, hypothesizing the etiology and mechanisms involved. He demonstrated that DOMS was a result of structural damage to the muscle and/or connective tissue and to a reduction in muscular performance [124]. This reduction in performance may result from a reduction in voluntary effort due to the sensation of soreness and/or a lowered inherent capacity of the muscle to produce force [101]. Therefore, DOMS is also accompanied by a significant decrease in force-generating capacity of the muscle resulting in eccentric strength decrements [125]. The time course for the development of soreness and loss of strength suggests little or no relationship between the two parameters [112]. Newham and colleagues [110] found that maximal voluntary force of the knee extensors had returned to normal by 24 hours after exercise while soreness at this time was most intense. Furthermore, Hough showed that the occurrence of delayed pain was directly related to the peak forces developed and to the rate of force development in rhythmic contractions, but not to the rate of fatigue [124, 126]. A reduction in maximum force production has been observed after eccentric exercise, as early as 1-hour post-exercise [113, 127]. Although Newham and colleagues have suggested that strength returns to pre-exercise levels within 24 hours [110], others have reported a return to baseline levels as long as 1 week [113]. Gleeson and colleagues [128] have reported that decrements in maximum isometric force (50% of normal) are greatest immediately following eccentric exercise, with recovery taking place by days 4-7. In addition, they report that a decrease in maximum dynamic power output (80% of normal) persists up to 4 days.

MacIntyre and colleagues [129] have reported a bimodal pattern of eccentric torque that occurs 0 and 20-24 hours post-exercise. This bimodal pattern was the first report demonstrating this pattern in humans. Faulkner et al [130] reported

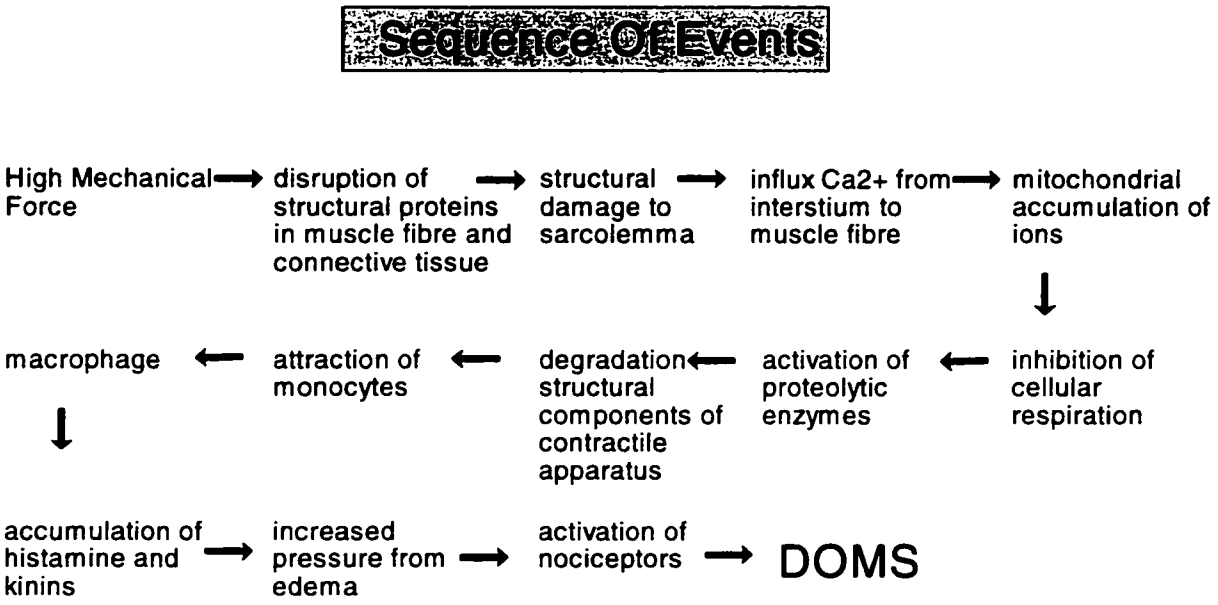
two declines in the muscle force in an animal model. They suggest that the initial decline in force may be a function of mechanical injury and fatigue (including myofibrillar disruption at the level of the Z-line), leading to an acute inflammatory response [129]. Faulkner and colleagues [130] further suggest that the second decline in force occurs in response to phagocytic activity at the site of the initial damage. This deficit in force does not appear to be related to the level of soreness since it occurs prior to the soreness and can remain for a greater period [131]. MacIntyre et al [132] have also reported that no relationship exists between the development of soreness and loss of muscle strength since the latter appears immediately after exercise. Therefore, this bimodal pattern of eccentric torque further grounds support for the theory that more than one mechanism is involved in exercise-induced muscle soreness [129, 130].

Theories Associated with DOMS

The pain and discomfort associated with DOMS has been studied extensively since 1902 and as a consequence, several theories have been suggested to explain this condition. Theories such as the *lactic acid theory* (lactic acid accumulation in the muscle), *muscle spasm theory* (originally described by DeVries [133] in 1961 that exercise causes ischemia which results in the production of a pain substance), *connective tissue damage theory* (rupture and damage of the muscle, predominantly the connective tissue), *muscle damage theory* (skeletal muscle damage the primary mechanism contributing to muscle soreness) and *inflammation* (tissue damage triggering an inflammatory response) as causes of DOMS have been postulated [105, 134].

Based on a review of the available published literature, it appears that DOMS is a result of two processes, a mechanical and biochemical process [102, 132]. A sequence of events representing these two processes during fatiguing exercise is outlined in Figure 2 [101, 126, 135].

Figure 2: Sequence of events associated with delayed onset muscle soreness, including mechanical and biochemical processes.



**adapted from Appell et al[126], Armstrong et al[101, 102]*

Stauber and colleagues [136] have suggested that the pain and inflammation associated with DOMS may be due to the swelling and disruption of the extracellular matrix. In contrast, Jones et al [137] have reported the stiffness to be a result of connective tissue damage. Pyne [138, 139] & Ebbeling and Clarkson [112] have suggested that mechanical stress (mechanical shear force production during exercise) and metabolic stress (disturbances in normal cellular metabolism provoked by exhaustive exercise) account for exercise-initiated damage to skeletal muscle fibres. Within the connective tissue of the muscles are myelinated group III (A-delta) and unmyelinated group IV (C) afferent receptors. The large myelinated group III fibers are believed to transmit "sharp" localized pain, whereas the group IV fibers carry "dull", diffuse pain. Therefore, it seems likely that the group IV receptors carry the sensation of DOMS since the pain is usually dull and diffuse, with the free nerve endings responding to mechanical and chemical (metaboceptors) as well as noxious stimuli (nociceptors) [112]. Bradykinin, serotonin and histamine may activate the free nerve endings of nociceptors to produce soreness and pain experienced after exercise [138]. Warhol et al [140] reported considerable disturbances in the contractile apparatus of the gastrocnemius muscle during competitive marathon running. Z-band streaming, myofibrillar lysis and contracture bands were noted upon histological examination. Furthermore, pathological changes occurred in the mitochondria, showing focal swelling and crystalline inclusions and the sarcolemma and sarcotubular system showed dilatation and disruption [126, 141]. Crenshaw and colleagues [103, 142] investigated whether DOMS of the vastus lateralis muscle was associated with elevated intramuscular pressure. Intramuscular pressure (IMP) is defined as the fluid pressure created by a muscle during contraction [142] and is correlated linearly with the force of contraction during isometric and isokinetic exercise [142]. Based on their findings, they first concluded that DOMS of the vastus lateralis muscle is associated with extensive intracellular swelling and with elevated IMP [103], however in a follow-up study, they reported that IMP was not an etiologic indicator of DOMS [142]. Newham [143] suggested that

intramuscular pressures are raised in some, but not all, painful compartments and even when raised, follow a different time course to the pain that appears.

Biochemically, a variety of clinical measures have been associated with DOMS. Muscle fibres contain proteolytic enzymes that are released following injury and initiate degradation of lipid and protein structures in the injured cell [144]. The presence of intramuscular enzymes in the blood has been considered to be indicative of damage to muscle fibres, particularly to the sarcolemma [101]. Numerous reports suggest the time courses of increased levels of plasma enzymes are similar to the time course of DOMS following exercise and the intensity of soreness and level of plasma enzymes are also correlated [107, 119, 145]. However, Donnelly et al [146] has suggested that muscle enzyme release and muscle soreness are unrelated. This suggestion was based on the findings that decline in muscle strength and in 50% endurance time did not differ between the first and second period (10 week gap) of the study, indicating 1) that the repeat bout effect for muscle enzyme release was not demonstrated and 2) muscle soreness reached the same level after both exercise bouts. In contrast, Armstrong et al [101] demonstrated in an animal model that elevations in plasma enzymes might occur simultaneously with exercise-induced necrosis of skeletal muscle fibers. Plasma enzymes such as myoglobin (18,000-Da heme-containing oxygen carrier protein of skeletal muscle cells [147]), hydroxyproline, creatine kinase (80,000-Da enzyme found in large concentrations in muscle tissue [147]), and hyperkalemia have been indicative of muscle injury [101]. Increases in serum activities of enzymes glutamic-oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH) and aspartate aminotransaminase (AST) has also been reported to reflect muscle fibre damage involving increased membrane permeability [138, 139, 148-151]. Mair and colleagues [152] have reported that an increase in myosin heavy chain (MHC) fragment plasma concentrations is demonstrated after eccentric exercise.

Creatine kinase (CK) is found almost exclusively in muscle tissue and is therefore considered the most common indicator of skeletal muscle damage [153]. The increase in serum or plasma CK activity after exercise is delayed and the extent of delay depends upon the type of exercise [112]. To explain this delay in CK release, Clarkson and Tremblay [154] have theorized that exercise-induced damage may cause an accumulation of Ca^{2+} resulting in; a) production of noxious stimuli such as bradykinin and histamine causing muscle soreness, b) muscle contractures leading to decreased range of motion, c) impairment of sarcoplasmic reticulum and mitochondrial functioning and d) activation of sarcoplasmic proteases resulting in loss of sarcolemmal integrity and delayed release of CK. Although CK has been widely used as a clinical marker for damage to the muscle, the relationship between the magnitude of CK release and histological evidence of the extent of muscle damage has not been established [155]. Smith [156] proposed that the most likely chemical stimulant for the induction of DOMS may be prostaglandin E_2 (PGE_2) that causes increased sensitivity of pain receptors and that invading macrophage have the capability of synthesizing PGE_2 [156]. Similarities in time course for increases in PGE_2 and DOMS ($p<0.05$), 24 hours after a bout of eccentric exercise was reported by Smith and colleagues [157]. Furthermore, Salminen and colleagues [158] have demonstrated in an animal study that *Indomethacin*, a non-steroidal anti-inflammatory drug that inhibits prostaglandin synthesis, had reduced damage to the muscle in exercised mice. On the other hand, Kuipers and colleagues [159] found that prostaglandins are not involved in an exercise-induced inflammatory response since flurbiprofen (cyclo-oxygenase inhibiting drug) did not have any effect on muscle soreness. Smith and colleagues [160] also studied whether there would be a reduction in total cholesterol levels in response to microtrauma induced by eccentric activity. This was founded on the notion that cholesterol is a component of the cell membrane and that cholesterol levels are temporarily reduced in response to trauma and post-surgery. Interestingly, the findings from this study showed a significant decrease in total cholesterol for both groups examined [160]. It has also been suggested that thiol proteases, such as

calpain, degrade structural proteins such as alpha-actinin, resulting in Z-line streaming and disorganization of the normal alignment of the myofilaments in conditions of altered metabolic and/or functional demands [132]. In an animal model involving the rat hindlimb muscle, Belcastro and colleagues [161] have reported increased calpain activity following level treadmill running, proposing that this increased activation of skeletal muscle calpain may result from increased intracellular calcium. Appell et al [126] showed that *nifedipine*, a calcium channel blocker, diminished exercise-induced muscle damage in a mouse model, supporting the notion that Ca^{2+} ions are implicated in the mediation of tissue damage.

Inflammation and Delayed-Onset Muscle Soreness

It has been suggested that muscle soreness is related to an inflammatory response. Inflammation is the body's normal response to an insult, such as an injury, infection or antigen. The purpose of this response is to clear and eliminate damaged tissue and microbial invaders, thus leading to tissue reparation. The main pathological feature of inflammation consists of leukocyte infiltration and exudation of plasma into the lesion in the early stage followed by proliferation of connective tissue including fibroblasts, which leads to the formation of granulation tissue.

Two sub-classifications of inflammation exist. First and foremost is the *acute* inflammatory process with *local* and *systemic* changes characterized by a rapid change in blood flow and accompanied by an immigration of neutrophils (histological hallmark of acute inflammation) and monocytes. Neutrophils are key nonspecific host defense cells responsible for phagocytosis of microbial, bacterial and viral pathogens [139, 162]. They play both efferent (phagocytosis and degranulation) and afferent (release of immunomodulatory molecules) roles in the immune response [139]. The cardinal signs of redness, swelling, heat and pain; *"rubor et tumor cum calore et dalore" (Cornelius Celsus)* are noted in the acute-local inflammatory phase [163, 164]. In addition, the induction of fever

(due to the production of IL-1, TNF, IFN-alpha) and the production of plasma proteins constitute the systemic reaction.

Chronic inflammation is characterized by the presence of lymphocytes and monocytes [132]. Monocytes and macrophages are primarily responsible for the removal of neutrophils and necrotic tissue [116, 132, 165]. After an insult occurs to the tissue, the body responds both at a vascular and cellular level. The former involves vasoconstriction (5-10 minutes), followed by vasodilation and increased vascular permeability [156, 166]. The latter response involves the interaction of various inflammatory mediators, mainly neutrophils and monocytes. A short time after the trauma occurs, circulating neutrophils dramatically increase in number, aggregating at the site of injury, reaching peak concentrations approximately 1-4 hours post-injury. This initial increase in neutrophils in the capillary bed of the injured tissue is due to the slowing down of blood flow within the capillary and the leak of plasma proteins from the capillary [132]. Smith et al [167] demonstrated a marked increase, above baseline, in neutrophil levels between 1 and 2 hours post-exercise. It has been demonstrated that during this period, neutrophils become intimately associated with the endothelial cells, upregulating adhesion proteins (CD11/CD18) that allow the neutrophils to role along the endothelial surface, become adherent and eventually migrating out into the tissue [132]. It is important to realize that only the presence of neutrophils in the interstitum or muscle is indicative of severe inflammation [132]. After this peak, the concentration declines rapidly and is followed by the migration of monocytes. This immigration rises in concentration and is maintained for 48 hours, after which the monocytes mature into macrophage. Macrophages play a pivotal role in the recovery process following exercise-induced muscle injury [132]. These adult macrophage remove necrotic tissue and foreign bodies [156, 166]. Monocytes/macrophage are responsible for the resorption of neutrophils in necrotic tissue and the sequestration of foreign material or antigens [132]. Furthermore, they are capable of producing a wide variety of cytokines in large amounts, thus contributing to the cytokine network. In addition, they play a pivotal

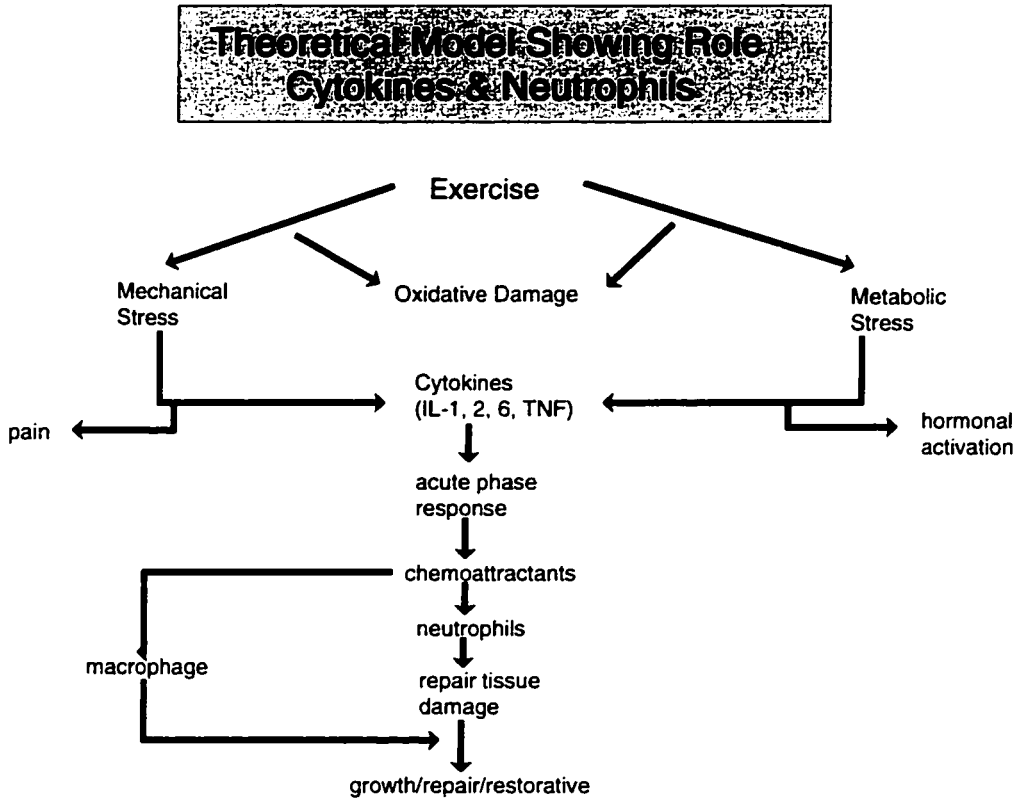
role in the specific response of T and B-lymphocytes to antigen [132]. There is a great deal of evidence suggesting the role of monocytes/macrophage during eccentric exercise, both in animal and human models.

Bendstrup [168] was the first to hypothesize that tissue damage resulting from intense exercise may trigger an inflammatory response and the time required for the response to occur explains the soreness delay [169]. It has been demonstrated that exercise-induced muscle injury triggers mobilization of some aspects of the inflammatory response [132], however the specific events initiating this seems somewhat unclear. It may be possible that the inflammatory response may be responsible for initiating, amplifying and/or resolving skeletal muscle injury [132].

It has been demonstrated that cytokines play a role in the immune response following strenuous exercise. However, Northoff et al [234] has reported that changes in cytokine levels observed in serum or plasma are always subtle, thus explaining significance in some studies while being borderline significant or undetectable in others. Cytokines are essential components of our defense and repair systems but also potentially harmful mediators of infectious and immunoinflammatory reactions [245]. Cytokines are released at the site of inflammation when there is a local response to an infection or tissue injury. They facilitate an influx of lymphocytes, neutrophils, monocytes and other cells into the tissue, and these cells participate in the clearance of the antigen and the healing of the tissue [187, 188]. Accompanying this local inflammatory response is a systemic response known as the acute phase response. The "inflammatory" cytokines produced as a result of this response include tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1) and interleukin 6 (IL-6). IL-1 and TNF increase after exercise and induce the release of a third cytokine, IL-6. Both IL-1 and TNF have proinflammatory effects while IL-6 has been cited to be restorative in nature, with anti-inflammatory and immunosuppressive effects [139, 231].

First introduced in 1980 by Weissenbach [235], IL-6 is a pleiotropic, "multifunctional" cytokine involved in the regulation of immune responses, the acute phase response (APR) and hematopoiesis [238]. Many different cells produce it after stimulation during infection, trauma or immunological challenge [231]. Its receptor system consists of two molecules: a ligand-binding 80-kDa molecule and a non-ligand binding signal transducer, gp 130, both of which were found to belong to the cytokine receptor family [232]. A theoretical model of the immunological and inflammatory responses to exercise and muscle damage showing the central role of cytokines and neutrophils in the repair of damaged tissue was proposed by Pyne [138, 139] [Figure 3]. Several authors have reported that eccentric exercise causing muscle damage is associated with an increase in serum IL-6 concentrations and this increase is significantly correlated with the concentration of creatine kinase in the days following exercise [150, 187, 188, 189]. The time course of cytokine production, the close association with muscle damage and the finding of increased IL-6 after intense exercise support the idea that during eccentric exercise, myofibers are mechanically damaged, thus stimulating the local production of inflammatory cytokines [187]. Rohde et al [188] have reported that eccentric exercise induced an increase in plasma concentrations of IL-6 by 570%, 2 hours post-exercise and return to pre-exercise levels by day 2. Bauer et al [233] have cited that elevated levels of IL-6 can be found as early as a few hours after the onset of a pathogenic event and may persist for only a few hours or up to a few days. Furthermore, Bruunsgaard et al [189] demonstrated that IL-6 levels increased five-fold and CK levels increased almost 40 fold, 4 days post-eccentric exercise. Furthermore, IL-6 seems to be the one cytokine that provides the most reliable results, being elevated shortly after strenuous exercise [234].

Figure 3: Theoretical model showing role of cytokines and neutrophils during exercise inducing damage to skeletal muscle[138,139].



*adapted from Pyne DB [138, 139]

Free Radicals and Exercise-Induced Muscle Damage

During intense exercise, whole body oxygen uptake can increase 20-fold above resting levels and in active muscle fibers, oxygen consumption may rise 200-fold [237, 238]. It has been estimated that 4-5% of the oxygen consumed during respiration is not completely reduced to water, instead forming free radicals [240]. A growing amount of evidence indicates that free radicals play an important role as mediators of skeletal muscle damage and inflammation [171]. Free radicals are chemical species with one or more unpaired electrons in their outer orbit making them highly reactive [112] since they strive to balance their unpaired electrons by combining with electrons with opposite spins in other substances [239]. Oxygen free radicals are increased during exercise as a result of increases in mitochondrial oxygen consumption and electron transport flux, inducing lipid peroxidation [239] [Table 9]. The cascade of lipid peroxidation is characteristic of inflammation in DOMS. Lipid peroxidation initiated by free radicals decreases the barrier function of cell membranes and may be associated with muscle fibre necrosis and enzyme release following damaging exercise [112]. During exercise, two potentially harmful free radical generating sources are semiquinone (in the mitochondria) and xanthine oxidase (in the capillary endothelial cells).

When the microcirculation is damaged, free radical formation may activate proteolytic enzymes [126, 229, 230]. Reactive oxygen species are also activated during ischemia/hypoxia and subsequent reperfusion/oxygenation in skeletal muscle [229]. Degradation of adenosine triphosphate (ATP) forms xanthine, which leads to a cascade effect on xanthine dehydrogenase, xanthine oxidase and uric acid, causing malfunctioning of the ion pumps and increasing intracellular levels of calcium [111, 229]. The resulting effect is the generation of oxygen free radicals, which induce disruption of phospholipid layers and lipid peroxidation [229].

Sjodin et al [171] have proposed that high intensity exercise increases the flow of oxygen through the skeletal muscles, causing metabolic stress and biochemical changes leading the skeletal muscle damage and inflammation [Figure 4].

Controversy also exists on the role of hyperbaric oxygen therapy in free radical-mediated tissue injury. Hyperbaric oxygen has been shown to enhance the antioxidative defense mechanisms in some animal models, but it has also been reported to increase the production of oxygen free radicals [193]. Several authors have reported that oxygen under certain conditions can generate highly reactive free radicals that mediate tissue injury and impair the process of wound healing while others have reported that hyperoxia increases the biochemical defense mechanisms against free radicals [193].

To assess the level of lipid peroxidation that occurs during oxidative stress on tissue, several reliable, analytical methods are available. The assessment of malondialdehyde (MDA), a product of lipid peroxidation, has become the most common technique to measure the degree of oxidative damage in biological systems [190]. Malondialdehyde is one of several low-molecular weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products [191, 192]. MDA, which may exist in a free form or as a complex with various tissue constituents, is formed during the oxidative degradation of some macromolecules, as a product of free radical generation by ionizing radiation in vivo, and as a by-product of prostaglandin biosynthesis [190]. MDA is formed during the last stages of the breakdown of endoperoxides formed during intramolecular rearrangements in the structure of polyunsaturated fatty acids [190]. Among the various methods to evaluate malondialdehyde, which include direct spectrophotometry or high-pressure liquid chromatography, the reaction with thiobarbituric acid (TBA) to form a colored adduct appears as a more rapid, inexpensive and sensitive technique [190, 191]. The sample under investigation is heated with TBA at low pH, and a pink chromogen (a TBA_2-

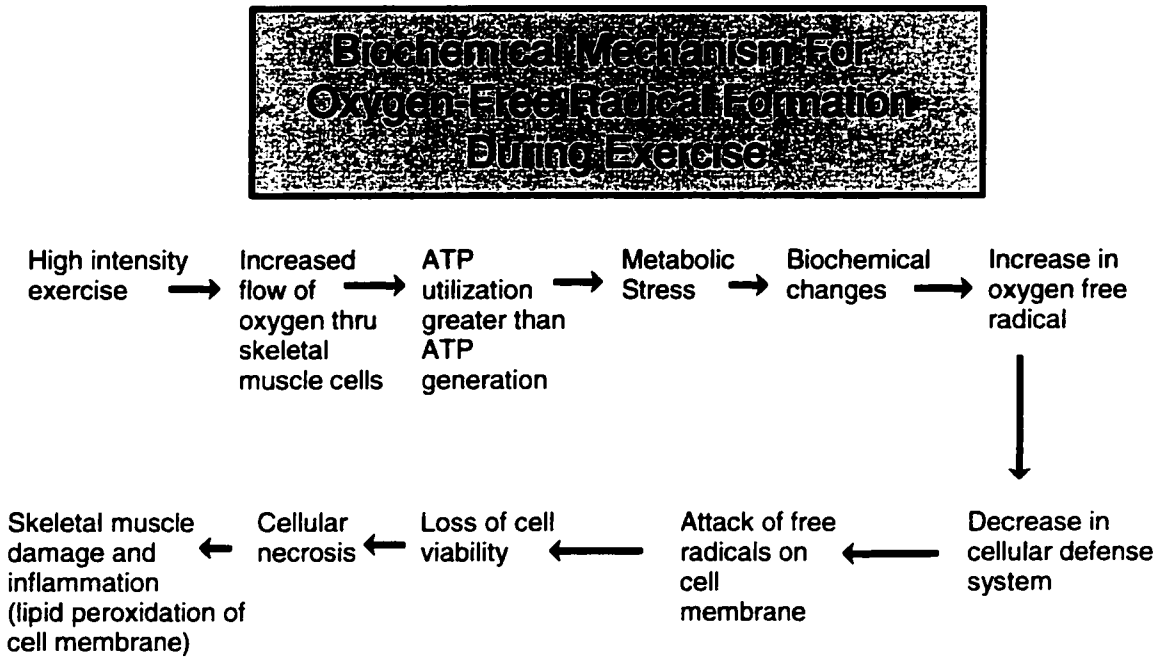
malondialdehyde adduct) is measured by its absorbance at or close to 532 nm or by fluorescence at 553 nm [236]. The fluorescence technique will be applied for our purposes in examining lipid peroxidation resulting from exercise-induced muscle damage.

Table 9: Mechanism by which exercise generates free radicals *

- Increases in epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically inactivated
- Production of lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl)
- Inflammatory responses to secondary muscle damage incurred with overexertion

Clarkson PM, et al 2000

Figure 4: Biochemical mechanism for oxygen-free radical formation resulting in skeletal muscle damage and inflammation during exercise



**adapted from Sjodin et al [171]*

Training and Adaptation Effect

A training and adaptation response has been reported in the literature. Schwane et al [145] suggested that training could reduce the magnitude of pathological alterations that occur after eccentric exercise. Newham et al [131] further suggested a few possible explanation for this training effect: 1) there is a change in the pattern of motor unit recruitment (i.e. either susceptible fibres are spared on second and subsequent occasion or more fibres are recruited and the force-fibre ratio is reduced), 2) there may be muscle fibre adaptation so that they become more resistant to the fatiguing and damaging effects of eccentric exercise and 3) the first bout of exercise had caused damage and destruction to a population of susceptible fibres, possibly those near the end of their life cycle. The time between exercise bouts would then be sufficient for regeneration thus making available a fibre population of high mechanical resistance [126]. Byrnes et al [172] and Nosaka et al [173] found that the effect of training on reducing indicators of eccentric exercise-induced muscle damage might be observed after only a single exercise bout. Similar results were also evident showing that training also reduces the increase in serum CK and myoglobin, upon repeat bouts of activity [112, 172, 173]. Byrnes et al [172] suggest that the mechanisms responsible for soreness and enzyme release are the same but the magnitude of the change between repeat exercise sessions can be altered by the performance on the first bout of work. In addition, Clarkson and colleagues [127] showed a decrease in response to a second bout of exercise after a 9 week separation between exercise while Byrnes et al [172] and Nosaka et al [173] reported that this prophylactic effect on the generation of muscle soreness and serum protein response may last up to 10 weeks.

2.6 The Role of Magnetic Resonance Imaging in the Detection of DOMS

Discovered in the 1940's, the phenomenon of nuclear magnetic resonance (NMR) has become a valuable instrument for the non-invasive study of muscle bioenergetics during exercise [176]. NMR can be used to produce high quality images using magnetic resonance imaging (MRI) and has been useful in

examining healthy and diseased skeletal muscle [174]. MRI, used to study protons, uses large volume radiofrequency coils and gradient magnetic fields that allow 2D and 3D images to be constructed [174]. The intensity of the generated images depends on the rate of relaxation of the protons in the sample area. Observing different types of nucleus relaxation, namely T1and T2 relaxation, can generate images of varying contrast [174].

T2-weighted proton images have been particularly useful in detecting differences in the chemical environment of cellular water, although T1 relaxation has also been investigated. Exercise changes the chemical properties of water molecules in muscle (e.g. increase in total water content [extracellular fluid]). The resulting effect is longer T1 and T2 relaxation times and brighter signals within muscle immediately following exercise [177-179]. Furthermore, the magnitude of these immediate post-exercise changes have been shown to be linearly related to exercise intensity [177-179]. Fleckenstein et al [179] found that after sports-related muscle injuries, delayed increases in muscle T2 relaxation times and signal intensities were demonstrated and that this outlasted all other indicators of muscle injury. This has also been reported in numerous other studies of eccentric exercise [180-183].

Nurenburg et al [182] has suggested that the sensitivity of MRI-guided biopsy for the detection of exercise induced muscle damage is by far more accurate in determining the extent and location of muscle injury than biopsies guided by DOMS. These investigators cite that there is a poor correlation between DOMS and CK and the extent of ultrastructural muscle injury but that there is a good correlation between signal intensity grades by MRI and the degree of ultrastructural damage. Several studies have used MRI to investigate changes in cross sectional area of muscle following a protocol of lengthening contractions [181, 183]. These studies demonstrate that the cross sectional area of muscle increases in a delayed manner with a time course similar to the changes in signal intensity and T2 relaxation in muscle.

Functional MRI refers to imaging not only the anatomy of a tissue but also the extent to which the tissue is involved in performing some task [226]. Muscle functional magnetic resonance imaging is used to compare the relative involvement of different muscles recruited during exercise. This method relies on the activity-induced increase in the nuclear magnetic transverse relaxation time (T2) of the muscle water, which is caused by osmotically driven shifts of fluid into the myofibrillar space [226]. In addition to imaging of whole muscle recruitment, muscle MRI may reveal changes in motor unit organization during disease [226].

The availability and sophistication of magnetic resonance imaging scanners have increased enormously during the last decade and has now become the method of choice for clinical imaging of most soft tissue pathologies, including sports-related injuries of muscle and joints [226]. Because of its noninvasive technique and its independence from ionizing radiation, researchers now use this imaging technique for basic and applied morphometric studies of human subjects. For the purpose of our study, MRI can easily measure the effects of exercise training on muscle and fat volume and the inflammation associated with delayed-onset muscle soreness.

2.7 Perceived Muscle Soreness and the Visual Analog Scale

Pain is very subjective in nature, thereby making it very difficult to objectively measure and quantify. The visual analog scale is one measure for quantifying pain [155, 195-197, 199, 201]. It consists of a 10 cm long line with marked endings indicating no pain at one end and extreme pain at the other end [APPENDIX B]. This line may be horizontal or vertical in nature with both showing a high correlation (r=0.99) [200]. In order to quantify and objectively measure pain, subjects are asked to place a mark along this line with respect to their level of pain at the time of measurement. This distance is then measured from one extreme of no pain to the area marked. Although a great deal of criticism exists in the literature with respect to the high variability inherent in this pain rating system, the visual analog scale has been found to bring greater

sensitivity and statistical power to data collection and analysis by allowing a broader range of responses than traditional categorical responses. It also removes the bias brought on by examiner questioning and allows for graphical temporal comparisons, thus minimizing bias and boosting statistical power [155, 198].

CHAPTER 3: METHODOLOGY

3.1 Experimental design

The experiment was divided into four stages:

- ***STAGE 1 (baseline/pre-exercise)***

  Evaluation period

  - Height
  - Weight
  - Perceived muscle soreness (VAS)
  - Eccentric strength (torque) assessment
  - Quadricep circumference
  - Blood analysis
    - Creatine Kinase
    - Interleukin 6
    - Malondialdehyde
  - Magnetic resonance imaging

- ***STAGE 2 (eccentric exercise protocol/DOMS)***

  Familiarization and warm-up period

  Exercise period (inducing muscle injury (DOMS))

- ***STAGE 3 (treatment)***

  Treatment period (HBO therapy)

- ***STAGE 4 (post-exercise evaluation – days 2-5)***

  Evaluation period (post-exercise)

  - Height
  - Weight
  - Perceived muscle soreness (VAS)
  - Eccentric strength (torque) assessment
  - Quadricep circumference
  - Blood analysis
    - Creatine Kinase
    - Interleukin 6
    - Malondialdehyde
  - Magnetic resonance imaging (days 1, 3 and 5)

Diagramatic representation of study design

SAMPLE	DEPENDENT VARIABLES	BASELINE Day1	DAY 2 4-h post-injury	DAY3 24-h post-injury	DAY4 48-h post-injury	DAY5 72-h post-injury
*Experimental Group (n=8)	Pain	x	x	x	x	x
	Strength	x	x	x	x	x
	Quadricep Circumference	x	x	x	x	x
**Control Group (n=8)	Blood (CK, IL-6, MDA)	x	x	x	x	x
	MRI	x		x		x

* Experimental Group : HBO treatment received days 2, 3, 4, 5
** Control Group: Received sham treatment on days 2, 3, 4, 5

3.2 Subjects

Sixteen female volunteers between the ages 18-40 participated in this study. Subjects were recreationally active with no weight training, running, or team sports as part of their physical regimen. Any previous experience with eccentric exercise would have caused adaptation of the muscle such that the muscle would have been more resistant to the effects of subsequent bouts of intense exercise. Subjects did not engage in physical activity for more than 3 hours a week and had to have met both inclusion and exclusion criteria to participate. Exclusion from participation in the study included athletes who actively weight train, run, jog, play in team sports, and/or ski. These activities involve repetitive eccentric loading of the quadriceps and therefore the eccentric exercise would not have produced the desired effect of DOMS. In addition, individuals who had experienced delayed-onset muscle soreness to their quadriceps in the previous three months prior to participating or who had a past history of severe joint injury, arthritis or other chronic illnesses were excluded. Subjects taking analgesics or prescription drugs were also excluded.

Contraindications to hyperbaric oxygen were also assessed (HBO contraindications: diabetes, lung cysts, epilepsy, upper respiratory tract infections, pregnancy, fever). After recruitment to the study, the subjects who met the above criteria were then required to carefully read and sign a consent form and fill out a questionnaire that was approved by the University of British Columbia Clinical Ethics Committee for research involving human subjects (APPENDIX A).

3.3 Procedure

The research protocol was a randomized double-blind design. Subjects were brought into the Allan McGavin Sport Medicine Clinic where they were screened and examined thoroughly to ensure that all the inclusion and exclusion criteria had been met and that they were not at any risk by undergoing HBO treatment. The subjects were then randomly assigned to one of two groups: a control (N=8)

and experimental group (N=8) and blinded to their specific treatment and group assignment. Eccentric torque (strength), perceived muscle soreness and quadricep circumference measurements were taken at baseline (Day 1) and after each of the four treatment sessions (i.e. Days 2-5). Blood samples were collected by antecubital venipuncture for the purpose of serum creatine kinase, interleukin-6 and malondialdehyde assessment at baseline (Day 1), 4-hours post-exercise (Day 2) and each day following treatment sessions (Day 3, 4, 5). Magnetic resonance images were collected at baseline (Day 1), 24-hours post-exercise (Day 3) and 72-hours (Day 5) post-exercise.

STAGE 1

Initially subjects were given a brief explanation of the experimental process. The subjects were then evaluated pre-exercise for perceived muscle soreness, eccentric muscle strength (torque) and quadricep circumference. Blood samples were collected to assess levels for CK, IL-6 and MDA and an MRI was taken of their quadricep muscles (both dominant and non-dominant). The height and weight of each subject was also recorded daily.

Perceived muscle soreness

Subjects were requested to give a subjective rating of muscle soreness in their quadricep of the non-dominant leg. The testing occurred pre-exercise and immediately after each hyperbaric exposure on days 2-5. They were instructed to complete four deep knee bends and then rate the soreness they experienced during these squats. The ratings were completed on the visual analog scale (VAS). The VAS is a 10cm line with *"no pain or discomfort" (e.g. 0)* at one end of the line and *"worst pain or discomfort" (e.g. 10)* at the other end. The subjects were given a form and asked to place a mark on the line as to where they felt the level of perceived muscle soreness (APPENDIX B). The VAS was used to record the subjects' perception of soreness of the quadricep on the exercised leg due to exercised-induced injury and not soreness experienced upon recollection of past injuries.

Eccentric strength

Isokinetic strength was measured using the KinCom Dynamometer. The KinCom Dynamometer (Chattecx Corp., Chattanooga, Tennessee) is a hydraulically powered, computer controlled exercise-testing device. This apparatus was used to measure and record the eccentric torque (Newton – metres, Nm) of the quadricep muscle as well as create the exercise-induced muscle soreness (DOMS) in the subjects.

Subjects were first instructed to ride a stationary bike for 5 minutes followed by a stretching exercise (lunges) before being seated on the exercise equipment. The subjects were then instructed to sit on the isokinetic dynamometer, with their hips at 80°, their back supported and their pelvis stabilized on the bench with strapping (fastening all three seatbelts (waist belt, left shoulder and right shoulder belt) to limit movement during the testing protocol). The researcher set the lever arm length of the KinCom dynamometer for each subject to 75% of the length from the head of the fibula to the lateral malleolus with the lateral joint line of the knee in alignment with the center of the rotational axis point of the machine. Securing the test leg on the upper third of the quadricep and the lower leg by a tight Velcro strapped shin pad ensured stabilization and limited the movement of the subjects' leg during the exercise protocol. They were further instructed to hold the sides of the seat for additional stability. The angular velocity was set at 30° through a range of 60° at a long muscle length (110 – 35° of knee flexion).

The subjects were then given a practice trial consisting of three submaximal and one maximal contraction (both concentric and eccentric contractions), followed by four maximal test contractions. A 2-minute rest period occurred between the practice and test contractions, with the practice serving as the warm-up for each test session. For the purpose of this study, only eccentric torque of the knee extensors was collected. The baseline mean torque value was collected from the average of three maximal efforts of the four repetitions.

Quadricep circumference

The measurements of quadriceps circumference were taken before the eccentric exercise protocol and after each treatment sessions using a standard anthropometric Gulick measuring tape (JUZO®). The same Gulick measuring tape was used throughout the entire testing protocol and the same side of tape was used consistently (i.e. the centimetre side of the tape would be on the superior side of the quadricep). Established landmarks were identified at the 10 and 20cm point above the superior border of the patella, as the subject lay supine on an examining table. A permanent, waterproof pen mark was placed at these two points so that the investigator could measure the same points throughout the testing period and ensures accuracy over the five days. This mark was reinforced on a daily basis. A mean of two measurements was obtained during every testing period. This measurement was used to evaluate changes in the circumference of the quadricep muscle, indicating the presence of edema.

Blood analysis

Thirty minutes after each treatment session, subjects were driven to St. Paul's Hospital (SPH) where twelve millilitres (ml's) of blood was withdrawn each day, during the five day testing period. Trained hospital laboratory technicians collected blood samples by standard antecubital venipuncture. Six milliliters of blood was collected in an SST *(serum separator)* tube for CK and MDA analysis and the remaining six milliliters was collected in an EDTA *(ethylene diamine tetra-acetic acid)* tube for IL-6 analysis. The blood samples were then spun to isolate the plasma, separated in 1.5 ml cryotubes and frozen at –70°C.

Analyses of all blood measurements were conducted at the phlebotomory laboratory at St. Paul's Hospital and Vancouver Hospital & Health Sciences Centre. The levels for each parameter (i.e. CK/MDA/IL-6) were carefully quantified and measured by highly qualified technicians in the laboratory as well as using state-of-the-art measurement devices (e.g. instruments measuring reflection densities, fluorescence, etc.).

Quality control was maintained for all blood analyses in the study. This allowed the investigator to be certain that the assay was reproducible and values obtained were consistent on a day-to-day basis. Calibration of instruments used in the laboratory is conducted every six months or when changes occur to the slide generator (reagent slides of the instrument). Adjustments to the instrument are made internally by the computer operating system. *Internal Quality Control* (IQC) and checks are carried out three times a day and are subsequently monitored on a monthly basis. Evaluations are sent out for comparison with other laboratories worldwide to further ensure reliability and validity of results. *External Quality Control* (EQC) is monitored by CEQAL®, which is mandated by the BC Medical Association, Department of Diagnostic Accreditation Program. Subscribers from Canada and the United States provide samples every 2 months and evaluations are processed with the results, including statistical analyses, being reported. Finally, the laboratory is fully accredited, with a five-year accreditation period. To ensure reliability and validity of MDA samples, a standard curve was constructed prior to assay analyses. Using a standardized curve ensured good reproducibility and precision of results on a daily basis. All samples were analyzed in a batch to further ensure reliability of the results. In addition, every subject served as their own control to examine changes of malondialdehyde levels post-injury, therefore showing lipid peroxidation changes over the five-day treatment period.

Creatine Kinase

Analysis of CK activity in serum plasma was conducted using the *Vitros* Chemistry Calibrator Kit 3™ and the *Vitros* CK Slide. The Vitros CK Slide is a dry, multilayered, analytical element coated on a polyester support. An 11 *u*L drop of sample is deposited on the slide and evenly distributed by spreading the layer to the underlying layers. This layer contains N-acetylcysteine (NAC) to activate CK without pretreating the sample. When the sample is deposited on the slide, creatine kinase catalyzes the conversion of creatine phosphate and ADP to creatine and ATP. In the presence of glycerol kinase (GK), glycerol is

phosphorylated to L-α-glycerophosphate by ATP. Oxidation of L-α-glycerophosphate to dihydroxyacetone phosphate and hydrogen peroxide occurs in the presence of L-α-glycerophosphate oxidase (α-GPO). Finally leuco dye is oxidized by hydrogen peroxide in the presence of peroxidase to form a dye. Reflection densities are monitored during incubation and the rate of change in reflection density is then converted to CK enzyme activity.

Interleukin-6

Serum IL-6 was analyzed using a standard ELISA kit obtained by *ChemiKine™*. This kit is a sandwich enzyme immunoassay (EIA), which measures the "free" forms of the cytokine IL-6. With this assay system, pre-coated mouse monoclonal antibodies generated against human IL-6 are used to capture human IL-6 in a sample. Simultaneously, IL-6 specific rabbit polyclonal antibodies detect IL-6 in the sample. With the addition of goat anti-rabbit conjugated-alkaline phosphatase (which binds to the rabbit anti-human polyclonal cytokine antibody), followed by the addition of the supplied color generating solution, the amount of IL-6 is detected.

Malondialdehyde

Finally, the analysis of serum plasma for MDA levels was completed using the standard TBA assay and fluorimetric analysis. Tetramethoxy propane was diluted with ethanol. An aliquot (250 *ul*) with distilled water was treated with 1.5 ml of 20% trichloroacetic acid (TCA) and then mixed with 1.5 ml of 0.67% thiobarbituric acid solution. The mixture was heated for 30 minutes and then spun at 2500 rpm for 10 minutes. Relative fluorescence intensity of the reaction product was measured at 515 nm excitation and 553 nm emission.

Magnetic resonance imaging

All scans were performed on a 1.5 tesla Siemens Symphony MRI system. The patients were positioned supine, and centered on the MRI scanning table, with the legs adducted. Coronal and axial scout images were obtained for initial

localization. Axial images were obtained from the level of the lesser trochanter to the superior pole of the patella. The field of view included both thighs. The positioning and slice locations were identical for all subjects and scans. The body coil was used for signal reception. T2 relaxation time and Short Tip Inversion Recovery (STIR) images were assessed. Surface coils of the radiofrequency system (i.e. body array coil) for magnetic resonance imaging were tested and rotated on a daily basis to maintain quality control for this measurement.

The pulse sequences were axial T2, axial STIR and coronal STIR. The axial T2 images used a TR 5500 msec, TE 110 msec, echo train 11, matrix 256 by 256, 10 mm slice thickness, 5 mm slice gap, one signal averaged. The axial STIR images used a TR 4300 msec, TE 30 msec, TI 160 msec, echo train 18, matrix 256 by 256, 10 mm slice thickness, 5 mm slice gap, one signal averaged. The coronal STIR images used the same parameters as the axial STIR images, except that the slice thickness was 5 mm, with no interslice gap.

The images were analyzed on a PC-based computer workstation using eFilm (eFilm 1.5.0 software version). This workstation software allows simultaneous viewing, windowing, and measurements to be performed on multiple slices from multiple imaging series. For measurement of muscle signal intensity, the images from the 24-hour post exercise scan were visually inspected to identify any areas of increased muscle signal (indicative of edema). The area of maximal edema was identified. An oval region of interest was manually traced around the area of maximal muscle edema. The region of interest was greater that 1cm in area, thus it represented at least 1 cc volume of muscle tissue. Care was taken to not include non-muscle tissues such as ligament, blood vessels, or fat in the region of interest. The corresponding area was identified in the opposite (dominant) leg, and in the identical anatomic location of the left leg on the first and third MRI scans. The signal intensity of the muscle was recorded. The ratio of signal intensity of the exercised (non-dominant)/signal intensity of the identical anatomic location in the dominant leg was calculated. The same process was repeated for

all three muscles : rectus femoris muscle, vastus intermedius and vastus lateralis muscle. A subjective score based on visual assessment was also recorded: 0 = no visible edema, 1 = minimal muscle edema, 2 = moderate muscle edema, 3 = marked muscle edema.

STAGE 2

Delayed-onset muscle soreness was induced on the KinCom Dynamometer in the quadricep muscle of the non-dominant leg. Subjects were seated on the exercise equipment as outlined in stage 1. The subjects were given a warm-up session to acquaint them with the eccentric exercise. This warm-up session consisted of one set of 10 repetitions at a submaximal effort. After this warm-up period, subjects were instructed to perform repeated eccentric contractions of their non-dominant leg (110^{o} - 35^{o} of knee flexion) at a slow speed (30^{o} per second) on the KinCom Dynamometer. The subject were instructed not to resist the concentric movement on the way up but to resist the machine's eccentric force on the way down. The exercise required the completion of 300 maximal voluntary eccentric contractions. The subjects completed 30 sets of 10 repetitions with each set beginning every minute for 30 minutes, allowing for a 15 second rest between each set. They received verbal feedback from the researcher (verbally encouraged to ensure that they are giving maximal effort) as well as biofeedback from the resistive force or force versus velocity curve displayed on the KinCom monitoring unit.

STAGE 3

Immediately after the exercise protocol and for the following 3 days, subjects were exposed to a hyperbaric environment (a total of 4 hyperbaric/normoxic exposures). Subjects were required to read a two-page information sheet on the hyperbaric unit and procedures for compression and decompression, prior to the first treatment session. Subjects were also required to try the available aviator-style gas mask to ensure a comfortable fit and to ensure that a tight seal was maintained between the mouth and nose. This mask was sterilized after each

treatment session to make sure that proper hygiene was maintained throughout the study period. Emergency equipment and clearing techniques were also explained (i.e. swallowing, valsalva manoeuvre, etc). Subjects were verbally instructed as to how they could decompress the chamber on their own and let themselves out if necessary. A microphone in the chamber was continually open and pointed out to the subjects to allow them to communicate any problems that they may experience during compression.

Control group

For each treatment session, the subjects were seated inside the HYOX monoplace chamber (Aberdeen, Scotland). The chamber was then compressed to a pressure of 1.2 ATA (an increase of 140 mm Hg). During compression, the subjects breathed the ambient air in the chamber. Once at a pressure of 1.2 ATA, the subjects were instructed to wear the gas mask, inspiring normoxic air (21% oxygen). The chamber was then reduced to barometric pressure for the remainder of the 60-minute treatment session. After 60 minutes, the subjects were instructed to remove the mask and again began breathing the ambient air of the chamber while decompression was initiated.

This increase in pressure to 1.2 ATA was sufficient to cause the subjects to experience the common tympanic membrane sensations associated with increasing ambient pressure. Therefore, it was unlikely for subjects to determine their group designation. The air was delivered through a series of regulators to the mask from high-pressure cylinders external to the chamber.

Experimental group

The compression and decompression procedure for the hyperbaric oxygen group was identical with the procedures for the control group, with the exception that this group was compressed to 2.0 ATA. Once at 2.0 ATA, the subjects in the experimental group received 100% oxygen, which was delivered to the aviator-style mask from high-pressure cylinders external to the chamber.

STAGE 4

The same evaluation was performed as stage 1 after each treatment session. All dependent variables were measured immediately post-HBO/normoxic treatment sessions (days 2-5).

3.4 Statistical Analysis

The study design involved 2 groups (experimental and control group) with a number of dependent variables (i.e. perceived soreness, eccentric strength, quadricep circumference, blood enzymes and MRI) that was measured on a number of repeated occasions. A two way (group x time) ANOVA was performed with repeated measures on the dependent variables, with the exception of perceived muscle pain (VAS scores). VAS scores were analyzed using a nonparametric test (Friedman test). For all statistical analyses, the significant level was set at $p<0.05$. Statistical analyses were performed using an IBM-compatible computer and SPSS 9.0 statistical software.

3.5 Statistical Power

Power for the study (0.76) was based on calculations of Cohen's Dc $(delta/s(1-r)^{1/2})$. Calculations were based on an alpha level set at 0.05, a 20% expected change, correlations of 0.6 and a standard deviation of 33% for the individual variables. These values were determined from previous literature and assumptions were based on clinical significance.

CHAPTER 4: RESULTS

Anthropometric Data

The mean physical characteristics of the 16 subjects who completed the study are listed in Table 10. No significant differences were detected between subjects in their height, weight and age ($p<0.05$).

Table 10: Physical characteristics for both groups (age, height and weight). Values reported as Mean ± SD.

	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)
Control (n=8)	25.25 ± 4.10	162.31 ± 6.03	57.0 ± 12.8
Experimental (n=8)	25.49 ± 4.24	160.90 ± 3.27	58.5 ± 10.1

Note: no significant differences between groups on any of the variables

Muscle Pain

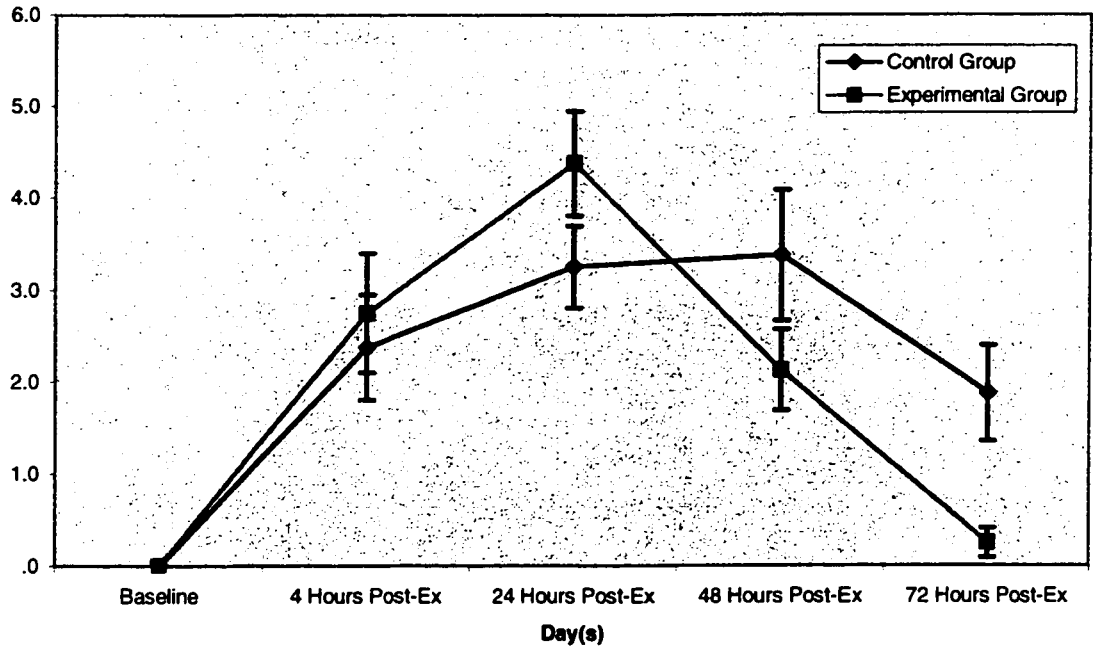
Results for the rating of perceived soreness of the non-dominant leg are illustrated in Figure 5. All groups showed a significant increase in soreness after the exercise protocol ($p<0.05$, $p=0.0001$) but there was no statistical difference between groups for treatment effects ($p=0.571$). A significant interaction effect between treatment and time was evident ($p<0.05$, $p=0.010$).

The control group showed an increase in pain from baseline, at 4 hours post-exercise, peaking at 24 hours and 48 hours post-exercise and decreasing 72 hours post-insult (APPENDIX C - Figure A). The experimental group had pain levels increase from baseline, 4 hours post-exercise, peaking at 24 hours and decreasing 48 hours and further decreasing 72 hours post-exercise (APPENDIX C - Figure B) [Table 11]. Both groups experienced less pain over time even though the peak time period for pain differed between groups.

Table 11: Average ratings of perceived soreness for the quadricep muscle of the non-dominant leg before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as visual analog score (1-10) ± SEM.

	Baseline (Day 1)	4 Hours Post-Exercise (Day 2)	24 Hours Post-Exercise (Day 3)	48 Hours Post-Exercise (Day 4)	72 Hours Post-Exercise (Day 5)
Control (n=8)	0.00 ± 0.00	2.38 ± 0.57	3.25 ± 0.45	3.38 ± 0.71	1.88 ± 0.52
Experimental (n=8)	0.00 ± 0.00	2.75 ± 0.65	4.38 ± 0.57	2.12 ± 0.44	0.25 ± 0.16

Figure 5: Average rating of perceived soreness for the quadricep muscle of the non-dominant leg, according to the visual analog scale (range 1-10), before (baseline) and after hyperbaric/normoxic exposure.



Eccentric Strength

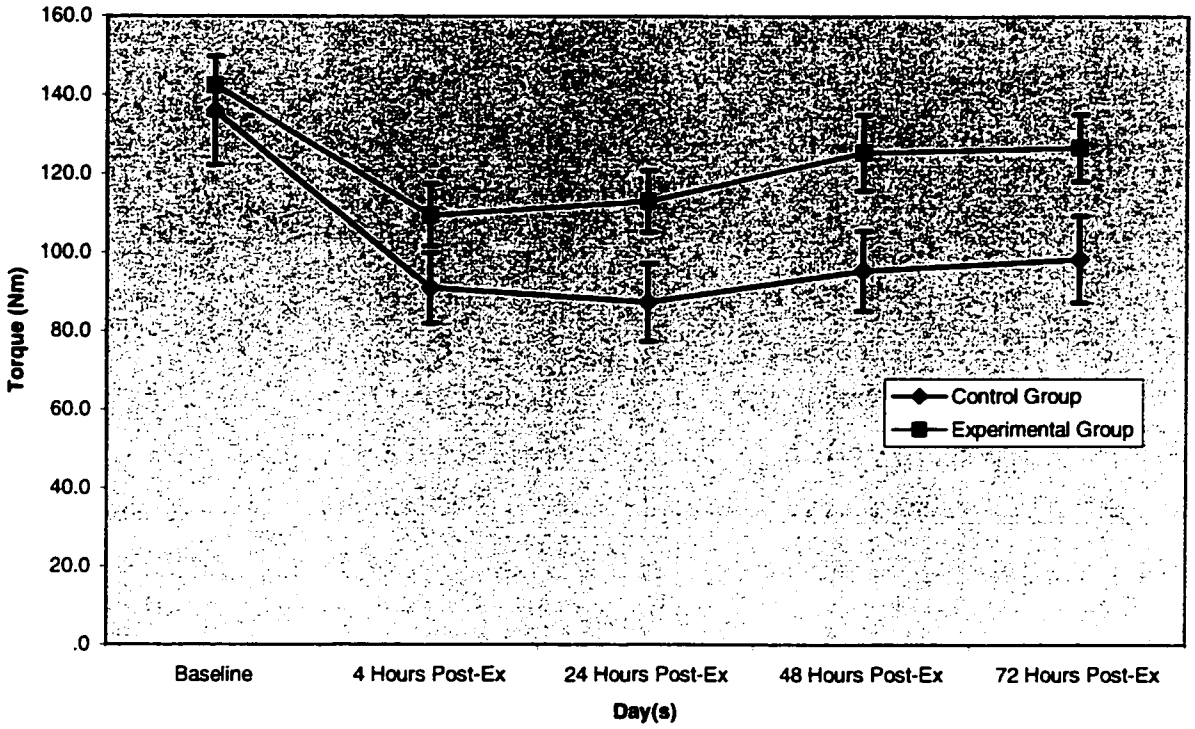
Analysis of the mean eccentric torque indicated that there were significant differences within the groups ($p<0.05$, $p=0.0001$); however, there was no statistical difference between groups and there was no significant interaction effect ($p<0.05$, $p=0.102$; $p=0.100$) (Figure 6).

The control group showed eccentric torque to be significantly lower 24 hours post-exercise, with gradual recovery by day 5 (72 hours post-exercise) (APPENDIX C - Figure C). The experimental group demonstrated a decrement in eccentric torque at 4 hours post-exercise, followed by gradual recovery 24, 48 and 72 hours post-exercise (APPENDIX C - Figure D) [Table 12]. Both groups demonstrated immediate eccentric strength decrements, followed by a gradual pattern of recovery.

Table 12: Average maximal eccentric torque for the quadricep muscle before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Strength values reported as maximal torque (Nm) ± SEM.

	Baseline (Day 1)	4-Hours Post-Exercise (Day 2)	24 Hours Post-Exercise (Day 3)	48 Hours Post-Exercise (Day 4)	72 Hours Post-Exercise (Day 5)
Control (n=8)	136.0 ± 13.75	91.0 ± 9.02	87.25 ± 9.92	95.38 ± 10.13	98.38 ± 11.10
Experimental (n=8)	142.38± 7.44	109.50 ± 7.95	113.0 ± 7.82	125.38 ± 9.56	125.63 ± 8.49

Figure 6: Average maximal eccentric torque for the quadricep muscle, before the exercise protocol (baseline) and after hyperbaric/normoxic exposure.



Quadricep Circumference

Analysis of the quadricep circumference at the 10cm reference point above the superior portion of the patella indicated that quadricep circumference was significantly different within the groups ($p<0.05$, $p=0.005$); however, there was no significant difference between groups and there was no interaction effect ($p<0.05$, $p=0.815$; $p=0.939$) (Figure 7). At the 20cm point above the knee, quadricep circumference was not significantly different within the groups ($p<0.05$, $p=0.253$) and again there was no significant difference between groups. Also, there was no interaction effect ($p<0.05$, $p=0.677$, $p=0.676$) (Figure 8).

Both groups (control and experimental), for the 10cm measurement point, demonstrated a slight increase in edema at 4 hours post-exercise, peaking at day

3 (24 hours post-exercise), decreasing on day 4 (48 hours) and finally showing a further decrease on day 5 (72 hours) post-exercise (APPENDIX C - Figure E, F).

The 20 cm measurement for both groups was slightly more variable. The control group had a slight decrease 4 hours post-exercise, increasing 24 and 48 hours post-insult and further decreasing 72 hours post-exercise (APPENDIX C - Figure G). The experimental group showed a slight increase in circumference 4 hours post-exercise, decreasing below baseline 24 hours post-insult, increasing 48 hours and finally decreasing 72 hours post-exercise (APPENDIX C - Figure H) [Table 13].

Table 13: Average quadricep circumference measured at both the 10 and 20cm point above the superior portion of the patella. Measurements were taken before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Quadricep circumference reported as centimetres (cm)) ± SEM.

10 cm	**Baseline (Day 1)**	**4-Hours Post-Exercise (Day 2)**	**24 Hours Post-Exercise (Day 3)**	**48 Hours Post-Exercise (Day 4)**	**72 Hours Post-Exercise (Day 5)**
Control (n=8)	44.99 ± 1.93	45.10 ± 1.78	45.31 ± 1.71	44.82 ± 1.91	44.13 ± 1.86
Experimental (n=8)	45.42 ± 2.04	45.73 ± 1.99	45.96 ± 2.18	45.71 ± 2.05	44.79 ± 1.99
20 cm	**Baseline (Day 1)**	**4-Hours Post-Exercise (Day 2)**	**24 Hours Post-Exercise (Day 3)**	**48 Hours Post-Exercise (Day 4)**	**72 Hours Post-Exercise (Day 5)**
Control (n=8)	53.49 ± 1.96	52.97 ± 1.99	53.19 ± 1.99	53.21 ± 2.01	52.89 ± 2.01
Experimental (n=8)	54.49 ± 2.17	54.64 ± 2.28	54.38 ± 2.38	54.68 ± 2.28	53.91 ± 2.15

Figure 7: Average quadricep circumference (10 cm location), before eccentric exercise (baseline) and after hyperbaric/normoxic exposure.

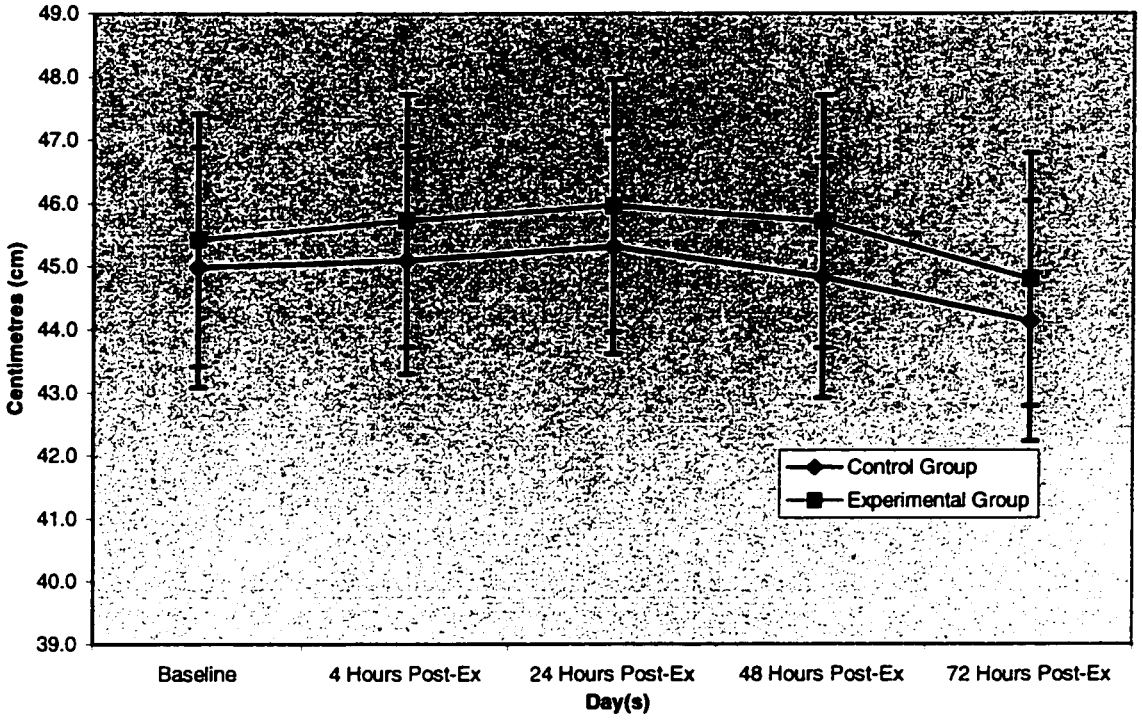
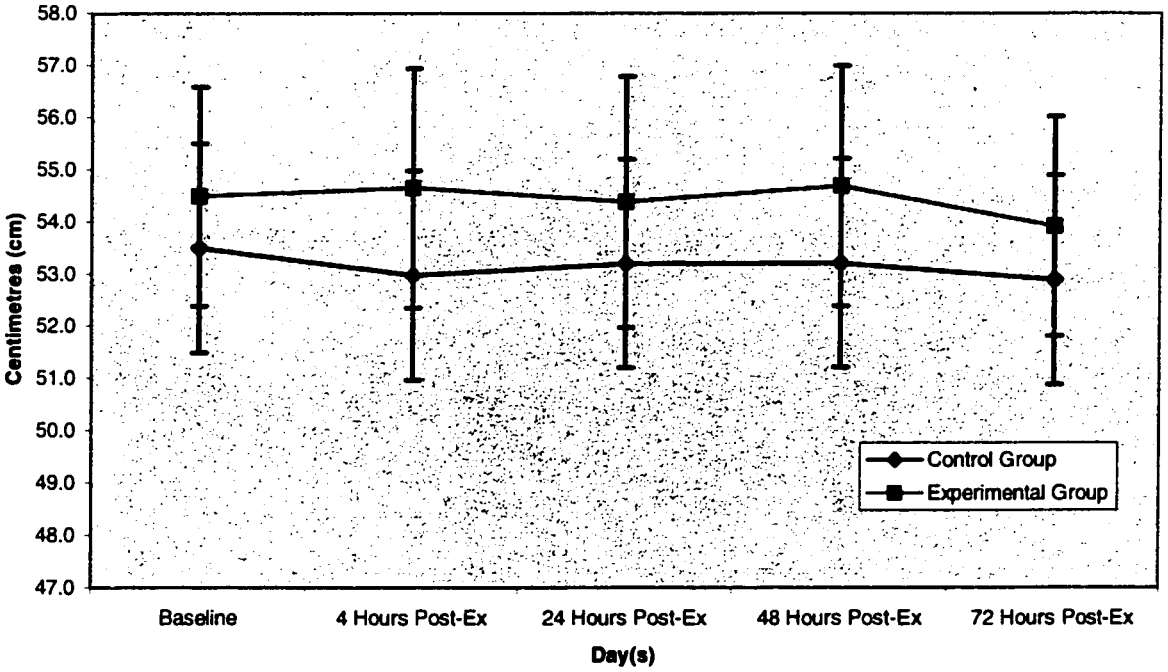


Figure 8: Average quadricep circumference (20 cm location), before eccentric exercise (baseline) and after hyperbaric/normoxic exposure.



Blood Enzymes

Creatine Kinase (CK)

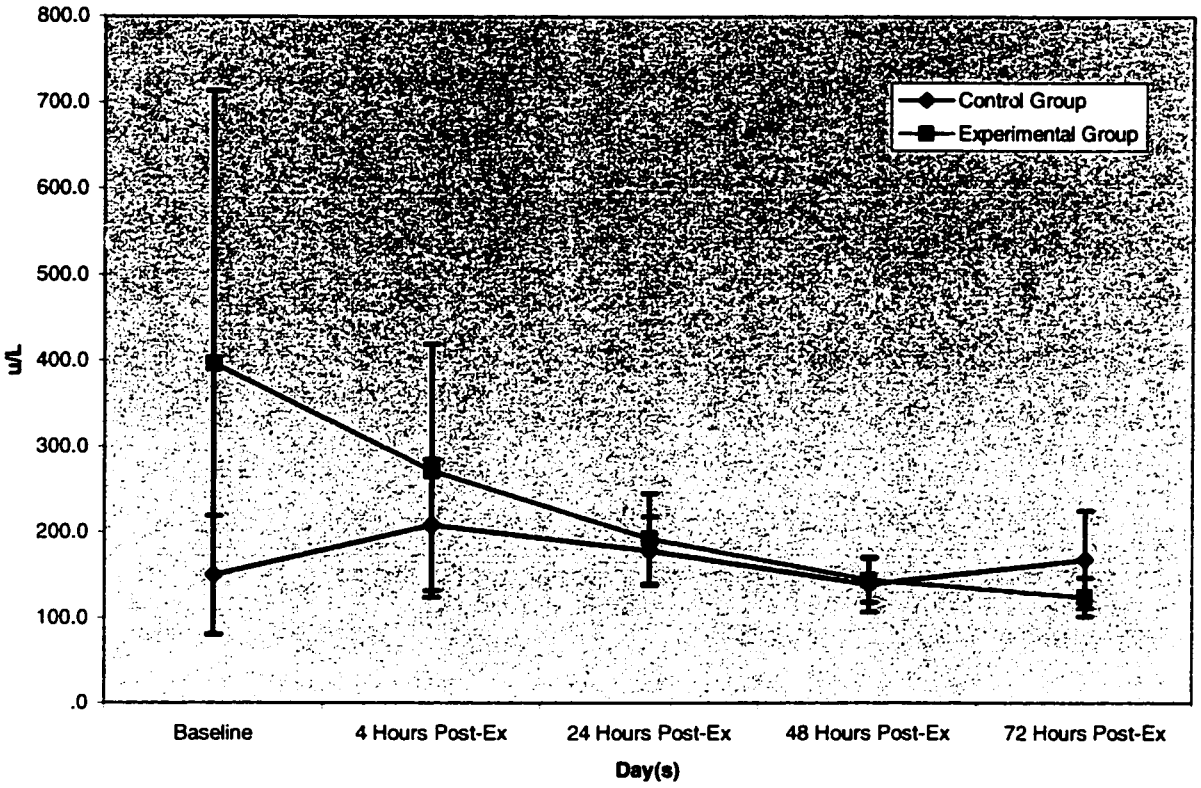
Analysis of the creatine kinase (CK) measurements indicated that CK was not significantly different within the two study groups ($p<0.05$, $p=0.538$). No significant difference was demonstrated between groups and there was no significant interaction effect ($p<0.05$, $p=0.647$; $p=0.570$) (Figure 9).

The control group demonstrated an increase in CK levels 4 hours post-exercise, followed by a decrease at 24 hours and 48 hours post-exercise, and an increase at 72 hours post-insult (APPENDIX C – Figure I). The experimental group showed a marked decrease from baseline at 4, 24, 48 and 72 hours post exercise (APPENDIX C – Figure J) [Table 14]. One subject had an unusually high CK response before the eccentric exercise protocol (>2500 *u*L). CK analysis was performed with and without this subject to examine whether the high variability introduced into the data by this subject was masking any significant statistical findings. Removing this subject from the statistical analysis did not alter the overall findings. Furthermore, removing this outlier from the data revealed a more stable curve, showing an increase from baseline at 4 and 24 hours post-exercise, followed by a gradual decrease at 48 and 72 hours post-injury [Table 14].

Table 14: Mean serum creatine kinase values before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. CK values reported as microlitres (μ/L) ± SEM.

	Baseline (Day 1)	4-Hours Post-Exercise (Day 2)	24 Hours Post-Exercise (Day 3)	48 Hours Post-Exercise (Day 4)	72 Hours Post-Exercise (Day 5)
Control (n=8)	150.50 ± 69.20	208.13 ± 76.76	178.58 ± 39.85	139.13 ± 32.22	168.13 ± 56.93
Experimental (n=8)	396.50 ± 316.42	271.63 ± 147.45	192.63 ± 52.75	143.88 ± 25.89	124.50 ± 22.38
Experimental *(minus outlier)*	80.14 ± 7.27	125.43 ± 22.11	145.30 ± 26.87	120.57 ± 13.02	110.43 ± 20.10

Figure 9: Average creatine kinase (CK) levels, before (baseline) and after hyperbaric/normoxic exposure.



Malondialdehyde (MDA)

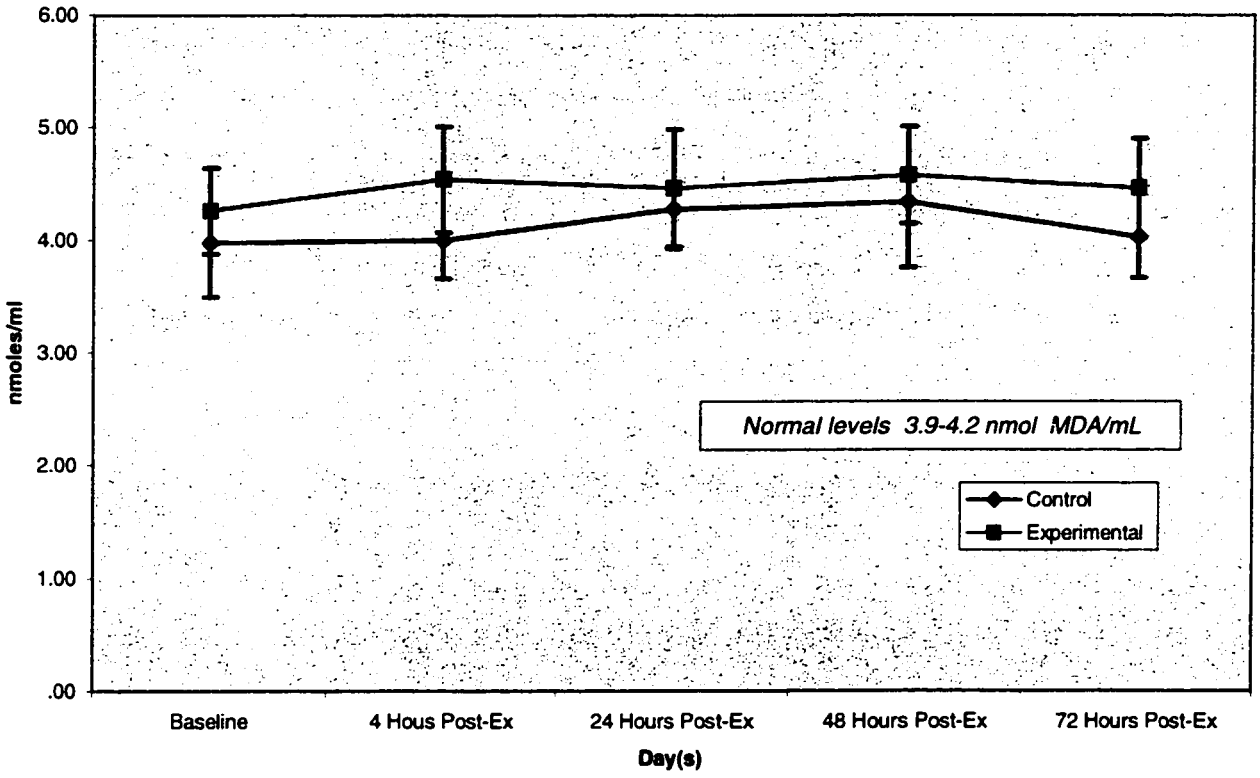
Malondialdehyde (MDA) results indicate no significant difference within the two study groups ($p<0.05$, $p=0.08$). No significant difference was demonstrated between groups for treatment effects and there were no significant interaction effects ($p<0.05$, $p=0.58$; $p=0.56$) (Figure 10).

The control group demonstrated very little variability between baseline values and post-exercise levels, increasing slightly days 3 and 4 and decreasing close to baseline by day 5 (APPENDIX C - Figure K). The experimental group, however, increased day 2, decreased slightly day 3, increased again on day 4 and slightly decreased by day 5. The values for the experimental group remained slightly higher than what was observed at baseline (APPENDIX C - Figure L) [Table 15].

Table 15: Mean malondialdehyde values before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. MDA values reported as nmoles MDA/mL ± SEM.

	Baseline (Day 1)	4 Hours Post-Exercise (Day 2)	24 Hours Post-Exercise (Day 3)	48 Hours Post-Exercise (Day 4)	72 Hours Post-Exercise (Day 5)
Control (n=8)	3.98 ± 0.48	4.0 ± 0.34	4.28 ± 0.35	4.34 ± 0.58	4.03 ± 0.36
Experimental (n=8)	4.26 ± 0.38	4.55 ± 0.47	4.46 ± 0.52	4.58 ± 0.43	4.46 ± 0.44

Figure 10: Average malondialdehyde (MDA) levels, before (baseline) and after hyperbaric/normoxic exposure .



Interleukin 6 (IL-6)

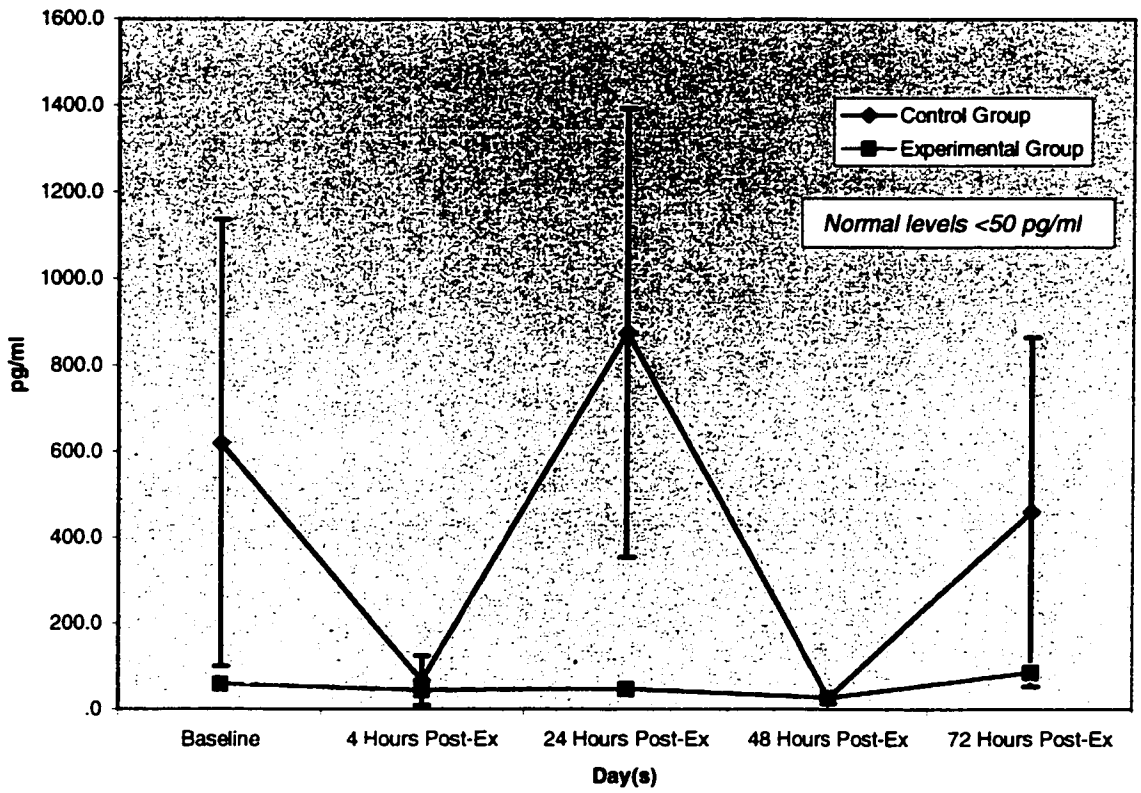
Interleukin 6 (IL-6) results indicate no significant difference within the two groups ($p<0.05$, $p=0.400$). No significant difference was demonstrated between groups for treatment effects and there was no significant interaction effect ($p<0.05$, $p=0.111$; $p=0.451$) (Figure 11).

Both groups demonstrated marked differences in the pattern of recovery from exercise-induced muscle injury and there was a great deal of variability. The control group fluctuated markedly; decreasing from baseline, 4 hours post-exercise, increasing above baseline 24 hours post-exercise, further decreasing at 48 hours to levels similarly to those seen at 4 hours post-exercise and increasing, although below baseline, by 72 hours post-insult (APPENDIX C - Figure M). The experimental group was more stable with less variability and fluctuations in IL-6 levels. From baseline, the IL-6 levels slightly decreased 4 hours post-exercise, remained at the same level 24 hours post-exercise, decreased further by 48 hours and increased above baseline levels by 72 hours post-exercise (APPENDIX C - Figure N) [Table 16].

Table 16: Mean interleukin-6 values before (baseline) and after (days 2, 3, 4, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. IL-6 values reported as pg/mL ± SEM.

	Baseline (Day 1)	4-Hours Post-Exercise (Day 2)	24 Hours Post-Exercise (Day 3)	48 Hours Post-Exercise (Day 4)	72 Hours Post-Exercise (Day 5)
Control (n=4)	618.13 ± 517.87	67.11 ± 57.83	873.19 ± 519.41	24.54 ± 11.71	459.26 ± 405.81
Experimental (n=4)	59.73 ± 21.98	44.80 ± 14.33	46.98 ± 20.94	26.08 ± 5.75	86.23 ± 21.03

Figure 11: Average interleukin-6 (IL-6) levels, before eccentric exercise (baseline) and after hyperbaric/normoxic exposure.



Magnetic Resonance Imagine (MRI)

T2-weighted images

Rectus Femoris

Analysis of the rectus femoris muscle using T2 weighted imaging revealed that this muscle was not significantly different within the two groups ($p<0.05$, $p=0.108$). No significant difference was demonstrated between groups for treatment effects and there was no significant interaction effect ($p<0.05$, $p=0.800$; $p=0.799$) (Figure 12).

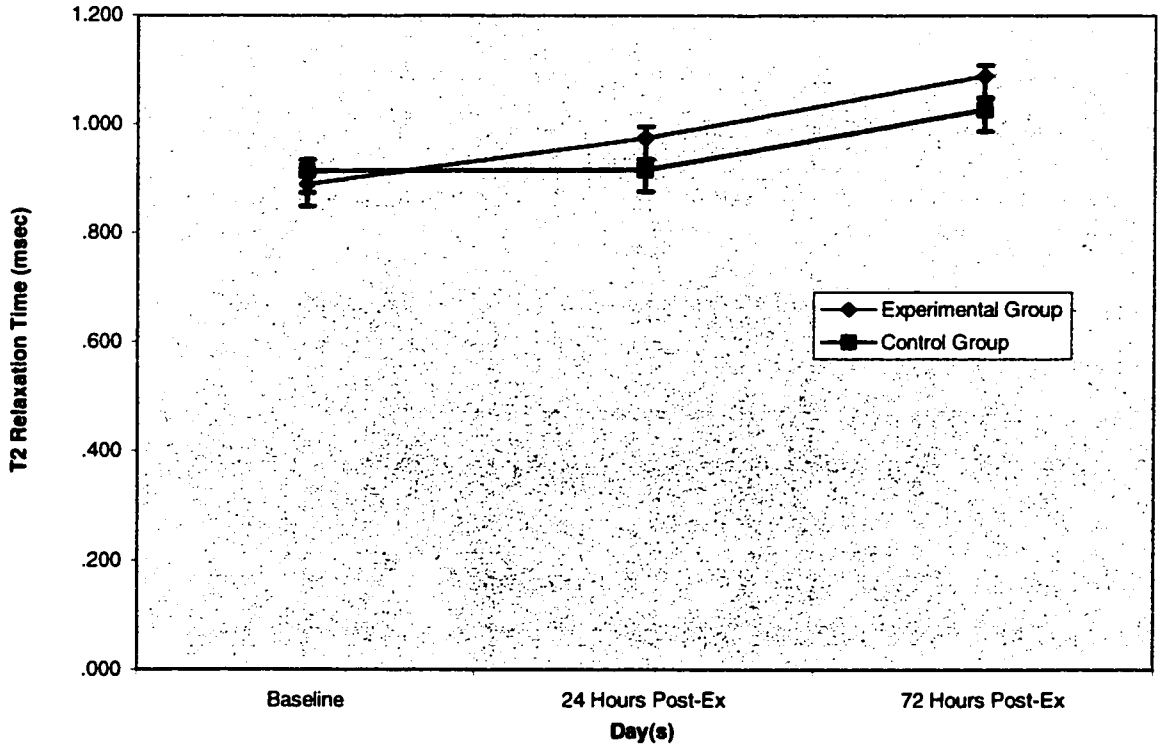
The control group demonstrated very little variability between baseline ratios and post-exercise ratios, revealing a slight increase by day 5 (APPENDIX C - Figure O). The experimental group also showed very little variability between days 1

through 5, however a slight increase was noted on day 3, followed by another increase on day 5 (APPENDIX C - Figure P) [Table 17].

Table 17: Average T2 relaxation times of the rectus femoris muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as T2 Relaxation Time (msec) ± SEM.

	Day 1 (Baseline)	Day 3 (24 hours post-exercise)	Day 5 (72 hours post exercise)
Control Group (n=8)	$0.91 \pm 7.02 \times 10^{-2}$	$0.92 \pm 4.34 \times 10^{-2}$	1.03 ± 0.10
Experimental Group (n=8)	$0.89 \pm 6.13 \times 10^{-2}$	0.97 ± 0.10	1.09 ± 0.19

Figure 12: Mean T2 relaxation times (msec) before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the rectus femoris muscle



Vastus Intermedius

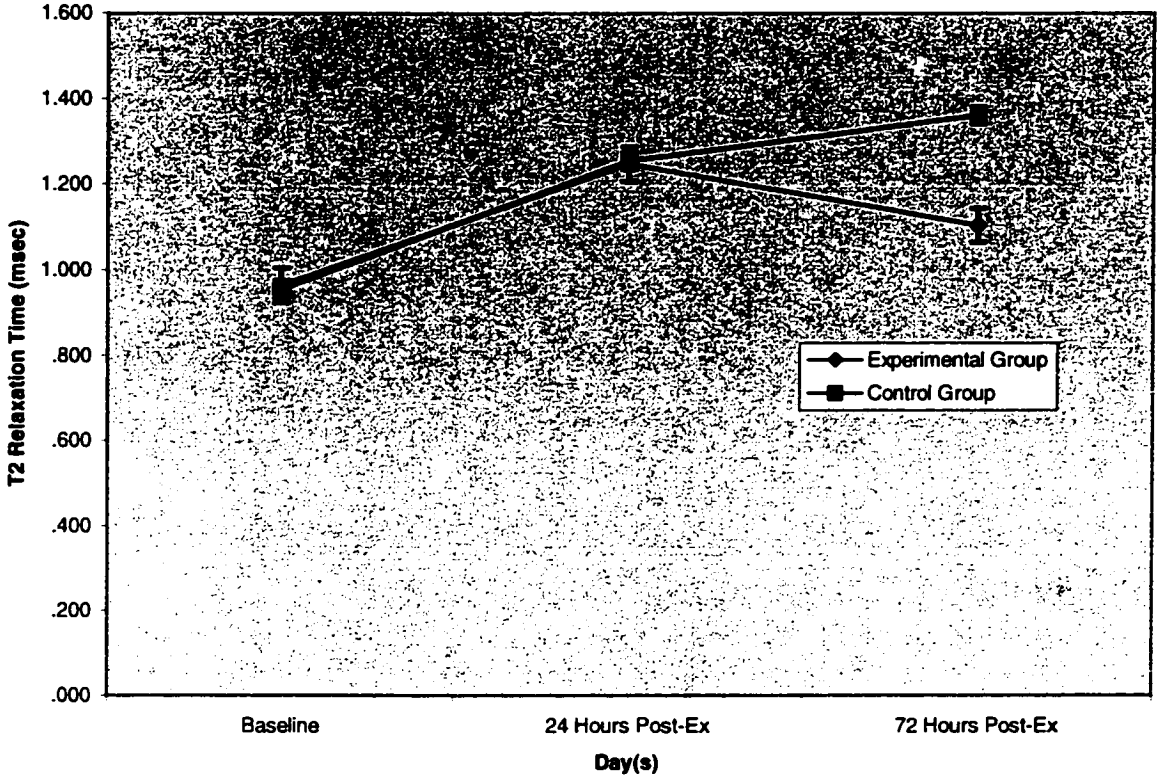
Analysis of the vastus intermedius muscle using T2 weighted imaging revealed that this muscle was significantly different within the two study groups ($p<0.05$, $p=0.007$) but no significant difference was evident between groups for treatment effects and group interaction effects ($p<0.05$, $p=0.361$; $p=0.259$) (Figure13).

The control group demonstrated an increase in signal intensity 24 hours post-exercise, followed by a further increase at 72 hours post-insult (APPENDIX C - Figure Q). The experimental group also showed an increase on day 3, but on day 5, a decrease was noted (APPENDIX C - Figure R) [Table 18].

Table 18: Average T2 relaxation times of the vastus intermedius muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as T2 Relaxation Time (msec) ± SEM.

	Day 1 (Baseline)	**Day 3 (24 hours post-exercise)**	**Day 5 (72 hours post exercise)**
Control Group (n=8)	$0.95 \pm 3.66 \times 10^{-2}$	$1.26 \pm 9.16 \times 10^{-2}$	1.36 ± 0.15
Experimental Group (n=8)	$0.96 \pm 4.85 \times 10^{-2}$	1.25 ± 0.13	$1.104 \pm 5.91 \times 10^{-2}$

Figure 13: Mean T2 relaxation times (msec) before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the vastus intermedius muscle.



Vastus Lateralis

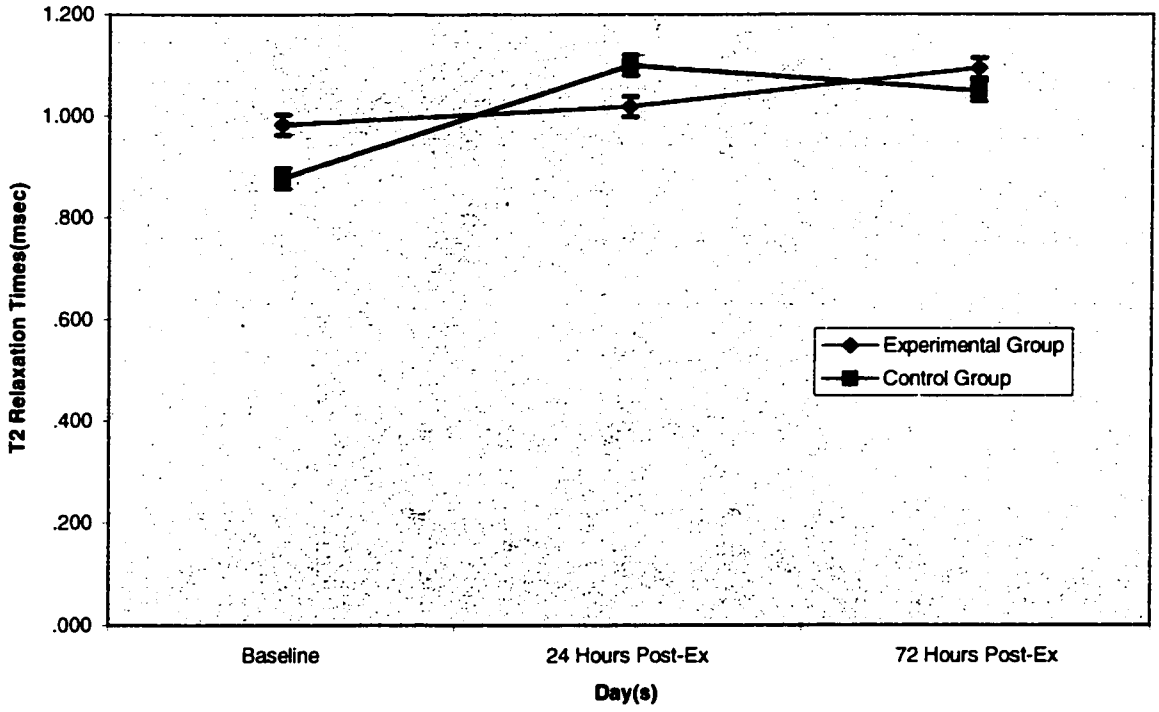
Analysis of the vastus lateralis muscle using T2 weighted imaging revealed that there was a significant difference within the groups for this muscle ($p<0.05$, $p=0.038$) but no significant difference was evident between groups for treatment effects and group interaction effects ($p<0.05$, $p=0.806$; $p=0.258$) (Figure 14).

The control group demonstrated an increase in signal intensity 24 hours post-exercise, followed by a slight decrease at 72 hours post-insult (APPENDIX C - Figure S). The experimental group also showed an increase 24 hours post-injury, followed by a further slight increase 72 hours post-insult (APPENDIX C - Figure T) [Table 19].

Table 19: Average T2 relaxation times of the vastus lateralis muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as T2 Relaxation Time (msec) ± SEM

	Day 1 (Baseline)	Day 3 (24 hours post-exercise)	Day 5 (72 hours post exercise)
Control Group (n=8)	$0.88 \pm 8.65 \times 10^{-2}$	$1.10 \pm 7.12 \times 10^{-2}$	$1.05 \pm 7.54 \times 10^{-2}$
Experimental Group (n=8)	$0.98 \pm 7.75 \times 10^{-2}$	$1.02 \pm 7.55 \times 10^{-2}$	$1.09 \pm 8.59 \times 10^{-2}$

Figure 14: Mean T2 relaxation times (msec) before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the vastus lateralis muscle.



STIR IMAGES

Rectus Femoris

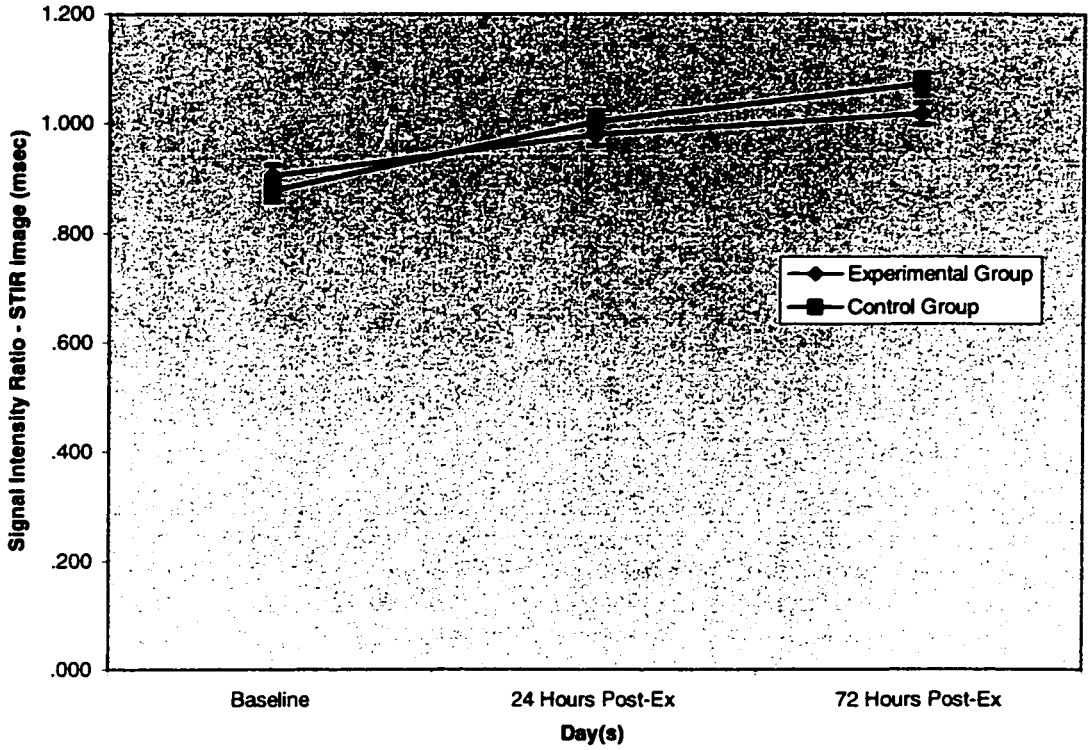
Analysis of the rectus femoris muscle using STIR imaging demonstrated that there was a significant difference within the groups ($p<0.05$, $p=0.018$) but no significant difference was evident between groups for treatment effects and interaction effects ($p<0.05$, $p=0.796$; $p=0.733$) (Figure 15).

Both control and experimental groups demonstrated a similar pattern over the 3 measurements taken over 5 days. The control group demonstrated an increase 24 and 72 hours post-exercise from baseline measurements (APPENDIX C - Figure U). The experimental group also showed an increase 24 hours post-injury, followed by a further slight increase 72 hours post-insult (APPENDIX C - Figure V) [Table 20].

Table 20: Average signal intensity ratio for STIR image of the rectus femoris muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as signal intensity ratio – STIR (msec) ± SEM.

	Day 1 (Baseline)	Day 3 (24 hours post-exercise)	Day 5 (72 hours post exercise)
Control Group (n=8)	$0.88 \pm 5.95 \times 10^{-2}$	$1.00 \pm 6.26 \times 10^{-2}$	$1.07 \pm 6.94 \times 10^{-2}$
Experimental Group (n=8)	$0.91 \pm 2.01 \times 10^{-2}$	$0.98 \pm 3.81 \times 10^{-2}$	$1.02 \pm 9.06 \times 10^{-2}$

Figure 15: Mean Signal Intensity Ratio for STIR image before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the rectus femoris muscle.



Vastus Intermedius

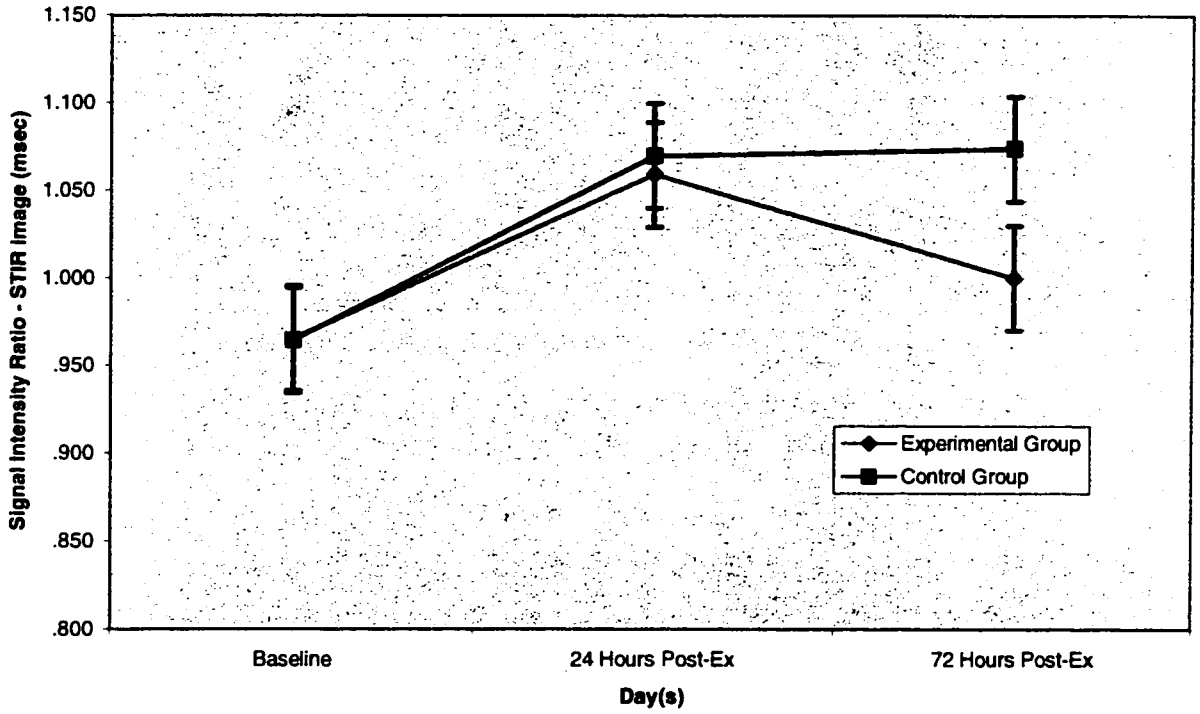
Analysis of the vastus intermedius muscle using STIR imaging demonstrated that there was a significant difference within the groups ($p<0.05$, $p=0.014$) but no significant difference was evident for between groups for treatment effects and group interaction effects ($p<0.05$, $p=0.580$; $p=0.451$) (Figure 16).

The control group demonstrated an increase in signal intensity 24 hours post-exercise, followed by a slight increase at 72 hours post-insult (APPENDIX C - Figure W). The experimental group also showed an increase 24 hours post-exercise, followed by a slight decrease 72 hours after injury (APPENDIX C - Figure X) [Table 21].

Table 21: Average signal intensity ratio for STIR image of the vastus intermedius muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as signal intensity ratio – STIR (msec) ± SEM.

	Day 1 (Baseline)	Day 3 (24 hours post-exercise)	Day 5 (72 hours post exercise)
Control Group (n=8)	$0.97 \pm 2.96 \times 10^{-2}$	$1.07 \pm 4.99 \times 10^{-2}$	$1.07 \pm 5.87 \times 10^{-2}$
Experimental Group (n=8)	$0.97 \pm 1.98 \times 10^{-2}$	$1.06 \pm 5.73 \times 10^{-2}$	$1.00 \pm 2.78 \times 10^{-2}$

Figure 16: Mean Signal Intensity Ratio for STIR image before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the vastus intermedius muscle.



Vastus Lateralis

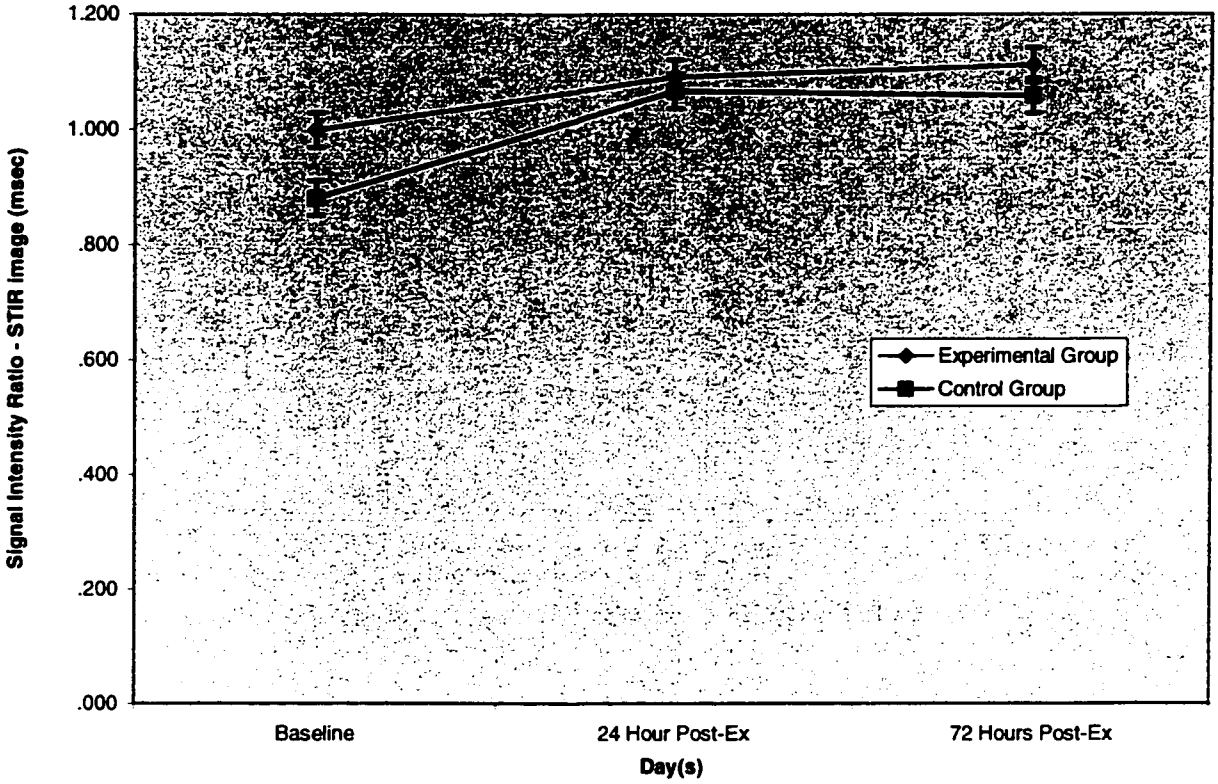
Analysis of the vastus lateralis muscle using STIR imaging demonstrated statistical significance within the groups ($p<0.05$, $p=0.004$) but no significant difference was evident between groups for treatment effects and group interaction effects ($p<0.05$, $p=0.265$; $p=0.560$) (Figure 17).

The control group demonstrated an increase in signal intensity 24 hours post-exercise, followed by a very slight decrease at 72 hours post-insult (APPENDIX C - Figure Y). The experimental group also showed a slight increase 24 hours post-exercise, followed by a further minimal increase 72 hours after injury (APPENDIX C - Figure Z) [Table 22].

Table 22: Average signal intensity ratio for STIR image of the vastus lateralis muscle taken before (baseline) and after (days 3, 5) the eccentric exercise protocol, following hyperbaric/normoxic exposure. Values reported as signal intensity ratio – STIR (msec) ± SEM.

	Day 1 (Baseline)	**Day 3 (24 hours post-exercise)**	**Day 5 (72 hours post exercise)**
Control Group (n=8)	$0.88 \pm 5.99 \times 10^{-2}$	$1.07 \pm 6.54 \times 10^{-2}$	$1.06 \pm 5.36 \times 10^{-2}$
Experimental Group (n=8)	$1.00 \pm 3.63 \times 10^{-2}$	$1.09 \pm 4.71 \times 10^{-2}$	$1.11 \pm 5.56 \times 10^{-2}$

Figure 17: Mean Short Tip Inversion Recovery (STIR) Ratios before (baseline) and after hyperbaric/normoxic exposure (days 3, 5) for the vastus lateralis muscle.



CHAPTER 5: DISCUSSION

The use of hyperbaric oxygen as a therapeutic modality is increasing among athletes, trainers, physiotherapists and other medical professionals. However, there is a paucity of scientific evidence to prove the effectiveness of using hyperbaric oxygen as a means of treatment. Previous studies examining the use of hyperbaric oxygen to ameliorate exercise-induced DOMS did not convincingly prove or refute the efficacy of hyperbaric oxygen in treating muscle and soft-tissue injuries. The aim of the present study was to investigate the influence of hyperbaric oxygen therapy on perceived muscle soreness, muscle strength, muscle edema and plasma enzymes.

The injury model that was used in this study, delayed-onset muscle soreness, allowed us to induce a quantifiable muscle injury and then monitor several variables over the course of recovery. This "gold-standard" injury model has been investigated in great detail for over 15 years and provides a good representation of muscle injury, damage and compromised force that may occur during sport and recreational activities [101-10, 105-106, 110-113, 127, 131]. However, caution should be applied when using this type of soft tissue injury model. It has been suggested by previous investigators [96, 246, 247] that although DOMS was created in the muscle of the subjects, the degree of muscle soreness may be at question, thus negating any beneficial effect the treatment modality may have in the rehabilitation process. This study elongated the muscle length of the quadricep to 110°-35° to further ensure high tension to the muscle fibres. In addition, visual estimation for the presence of edema was made 24 hours post-injury through magnetic resonance imaging. However, although every effort was made to ensure that muscle damage occurred to the quadricep, the degree of damage for this study is also questionable.

Females were used in the study to make the group as homogeneous as possible and to control for extraneous variables. Furthermore, sex differences in muscle fatigue have been reported in the literature, with females generally exhibiting a

greater relative fatigue resistance than males [252-253]. This phenomenon has been observed in a variety of muscles with the use of various fatigue protocols. Hicks et al [254] hypothesized that muscle mass, substrate utilization and muscle morphology may be mechanisms involved attributing to differences in fatigue resistance. Estrogen may also be implicated in modulating muscle fatigue. Estrogen receptors are found on vascular endothelial and smooth muscle cells [255]. Estrogens affect vascular tone indirectly by modulating release of endothelium-derived vasoactive factors and directly by modulating intracellular calcium in vascular smooth muscle cells [255]. Estrogens indirectly affect thrombotic events and inflammation by altering platelet aggregation and leukocyte adherence and migration, respectively [255]. Estrogens also influence production of mitogens which, when released at sites of vascular injury, affect vascular remodeling [255]. Therefore, one gender was selected to control for gender differences associated with fatigue resistance.

The study, in its entirety, was completed with 16 female participants. Although it was anticipated that the study would recruit 20 subjects, time constraints and the exclusion of a few individuals due to interruptions in treatment hindered this effort. Because of this decrease in sample size, power of the study was reduced from the pre-calculated 0.76, thereby limiting the ability to detect a treatment effect, given that an effect actually existed. This limitation of a relatively small sample size could therefore have had an effect on not attaining statistical significance in the study for the variables examined.

This double-blind study examined numerous variables. *Pain* and *strength* parameters were examined to demonstrate perceived muscle soreness and eccentric strength decrements, *MRI* and *quadricep circumference* were measured to indicate muscle edema that is demonstrable in muscle injury, and blood levels for *CK, IL-6, MDA* were examined to illustrate skeletal muscle damage, the cytokine response and lipid peroxidation. Through these measurements, the patterns of recovery could be seen between both groups and

therefore would allow us to determine whether the experimental group showed a faster course of recovery to baseline levels over the five day testing period in contrast to the control group.

Perceived muscle soreness

It was hypothesized that the muscle soreness experienced by the DOMS protocol would be reduced by hyperbaric oxygen therapy over the testing period. The results of this study do not support this hypothesis. Hyperbaric oxygen therapy did not significantly decrease muscle pain or soreness levels over the course of four treatments. These results contradict the findings of other reports indicating that HBO therapy successfully alleviated muscle soreness in athletes [1, 95].

Borromeo et al [95] used the visual analog scale to assess pain levels for acute ankle sprains over a course of three treatments. He demonstrated pain levels peaking at 3.25 and 2.6 for the HBO and control group, respectively, and decreasing to near baseline levels by completion of the third treatment. Harrison et al [246] and Mekjavic et al [225] used the visual analog scale to assess pain levels in their sample population. Their studies demonstrated peak ratings as high as 7 for perceived muscle soreness for both groups. Balnave et al [147] showed a rating of 6 for peak muscle soreness. Another study by Zhang et al [229] showed peak pain levels of 2.5 and 4.5 for the intervention and placebo, respectively. These studies all show ratings of muscle soreness ranging anywhere between 2.5 and 7. This is similar to the results of this study, which demonstrate that muscle soreness peaked in range between 3.5 and 4.5 for the control and HBO group, respectively.

The majority of previous studies cite perceived soreness to peak anywhere between twenty-four and forty-eight hours post-exercise [129, 147, 225, 246]. Similarly, this study supported these finding for both groups. The perception of muscle soreness peaked 24 hours after the high-force eccentric exercise protocol

for the experimental group while the control group peaked 48 hours post-eccentric exercise.

Several reasons may be postulated for the failure of the HBO treatments to alleviate muscle soreness. The findings of this study may be due to intersubject variability. Pain or perceived soreness remains a subjective variable in which the subject is asked to graphically display their perception of pain. Pain thresholds and tolerance levels are different among individuals [241]. The perception of soreness is very difficult to interpret as perceived muscle soreness is subjective in nature and its perception varies from individual to individual. Stewart [241] has cited that the perceived pain is a product of interpretation by the mind; the intensity felt can be increased or decreased by conscious and unconscious thought or emotions. The sensory component may be modulated by the subject's past experience where attitudes and psychological variables may influence description of the sensation [129]. Error is easily introduced as an individual may report more or less pain that what is actually felt. Less pain is usually the direction in which individuals follow in reporting pain [241].

The visual analog scale has been cited in previous literature as a reliable instrument in measuring pain or perceived soreness in subjects [15, 195-197, 199, 201]. However, by marking on a 10 cm line the level of pain felt can introduce error in accuracy of what is actually felt versus what is begin reported. Furthermore, frequency in sampling may also bias results. Subjects may have been aware of their previous response and perhaps subconsciously bias their following responses.

Newham et al [104] reported that pain and tenderness is usually localized to the distal third portion of the muscle, in the region of the musculotendinous junction where muscle pain receptors are most concentrated. In addition, pain associated with DOMS appears medially, laterally and then distally, eventually spreading to the center of the muscle belly by 48 hours [104]. Generally, however, pain is

evident throughout most of the affected muscle belly while no pain is experienced during rest [132]. All subjects in the study demonstrated a similar pattern of pain, with the musculotendinous junction to be the primary location of soreness. Furthermore, as expected, all subjects (both control and experimental groups) demonstrated no pain at rest but experienced discomfort during movement.

Another possible explanation for the lack of significant differences in pain data was that the stimulus in this study did not cause enough muscle damage and injury. Even though we increased the angle of muscle elongation to an 110° - 35° muscle range, perhaps there wasn't enough stimulus to induce muscle injury and damage to warrant sufficient DOMS to show significant results in treatment. The exercise protocol that was used in this study was one that has been tested by McIntyre et al [129]. These investigators have used this protocol on the non-dominant quadricep muscle to elicit and induce sufficient muscle soreness. Laurence [247], had suggested that in his study, at 95° – 35° flexion, the quadricep musculature may not be elongated enough to induce sufficient muscle soreness. Therefore, this study employed his suggestion of using 110° – 35° flexion to further elongate the quadricep muscle, thus ensuring that the stimulus would in fact induce sufficient muscle soreness. All subjects were carefully monitored during the exercise protocol and were encouraged to exert maximal effort on each contraction. There efforts were also observed on the visual display to see that they were not only maximizing their efforts but also maintaining this level consistently throughout the protocol. However, even though we ensured these efforts to induce muscle injury, perhaps the level of muscle damage was not sufficient enough to warrant DOMS.

Eccentric Strength

It was hypothesized that treatment with hyperbaric oxygen would return strength faster than the normal course of recovery. This however was not the case as there were non-significant findings between groups in terms of treatment and placebo sessions. Hyperbaric oxygen did not accelerate the injury healing

involved in restoring strength as neither the rate or magnitude of recovery of eccentric strength differed between groups. This contradicts the findings of Staples et al [96] who demonstrated significant results when analyzing mean torque data, thus showing a reduction in HBO vs. control group.

An initial decline in strength was apparent between both groups in the study immediately after the eccentric exercise protocol. This was followed by a gradual return of torque over four day, although these levels did not return to baseline (pre-exercise) by day 5. MacIntyre et al [129] have reported similar findings. In their study, torque measurements did not return to baseline for 6 and 7 days, respectively. The initial decline in strength corroborates other findings of strength decrements immediately following a high intensity exercise protocol [129, 130]. They suggest that the initial decline in force may be a function of mechanical injury and fatigue (including myofibrillar disruption at the level of the Z-line), leading to an acute inflammatory response [129, 130]. A second decline in strength (bimodal pattern) was seen with six of the sixteen subjects in this study. This corroborates findings in previous research suggesting that a bimodal pattern of eccentric strength is seen in both an animal model and humans [129, 130]. Faulkner and colleagues [130] further suggest that the second decline in force occurs in response to phagocytic activity at the site of the initial damage. This deficit in force does not appear to be related to the level of soreness since it occurs prior to the soreness and can remain for a greater period [131]. Previous studies have reported that there is no relationship between the level of soreness and the decline in muscle strength [129, 130, 131, 132]. Strength decrements are seen immediately post-exercise while soreness develops 24-48 hours post-exercise. Observing a bimodal pattern of eccentric torque, lends more evidence and support to the theory that more than one mechanism (i.e. mechanical, biochemical) is involved in exercise-induced muscle damage.

Both groups demonstrated gradual recovery over time, although individual variability in eccentric torque was evident among all subjects [APPENDIX C –

Figures D, E]. To explain this variability, several explanations may be worth introducing. First, it would have been worthwhile to observe the recovery pattern of all subjects for more than 4 treatment sessions. By only taking eccentric torque measures at post-exercise, 24,48 and 72 hours, we weren't able to follow the course of recovery over the previous cited 5-7 days recovery period for DOMS [132]. Furthermore, a learning effect could have played a role in the recovery pattern seen among subjects. Numerous other investigators have reported a training and adaptation effect during eccentric exercise [131, 145, 172, 173]. Nosaka et al [173] has suggested that a subsequent single bout of eccentric exercise may reduce indicators of muscle damage. Newham and colleagues [104, 110, 131, 143] have suggested that this may be caused by a change in motor unit recruitment pattern, muscle fibre adaptation and/or a regeneration of new mechanically resistant fibres resulting from damage and destruction to the original recruited fibres. The subjects could have become more comfortable and more experienced day by day as they performed the eccentric strength test. Although subjects were encouraged verbally by the investigator to produce maximal effort throughout the exercise protocol and isokinetic strength tests, and their efforts were monitored on the visual display, their energy level may have fluctuated daily. This would result in variability in strength levels throughout the course of the testing period.

Quadricep Circumference

Quadricep circumference was measured at the 10 and 20 cm point above the superior portion of the patella, over the course of the 5 days. The premise for this measurement was that during the strenuous eccentric exercise, muscle fatigue and injury would give rise to muscular edema in the quadricep muscle of the non-dominant leg. Edema is a result of the inflammatory process and could lead to increased pain and decreased range of motion.

The eccentric exercise-induced edema, as reflected in quadricep circumference, did not demonstrate significance between grcups. Slight increases in

circumference were demonstrated between groups at both the 10 and 20 cm mark, however not enough to attain statistical significance.

Evans et al [150, 165] suggest that evidence of swelling has ranged from increased circumference of the exercised muscle 24-48 hour post-exercise to ultrastructural evidence of post-exercise edema. Similarly, the results of this study demonstrated that peak swelling (i.e. increased quadricep circumference) at the 10 cm point occurred on day 3 (24 hours post-exercise) for both groups. Peak swelling at the 20 cm point was evident on day 4 (48 hours post-exercise) for both groups, however the measurements for days 2-5 for the control group remained lower than baseline. Mekjavic et al [225] also demonstrated increases in arm circumference on days 3 and 5, post-exercise.

Edema is a result of either an increase in vascular permeability of small blood vessels, or leakage of intracellular fluid into the extracellular space [131], resulting in tissue hypoxia, due to the increased oxygen diffusion distance from the capillaries to some cells, and the increased interstitial pressure around the capillaries due to the fluid accumulation [225]. It has been reported that muscle edema is caused by increased amounts of degraded protein components of the muscle and the release of protein-bound ions in damaged muscle cells [42, 165, 154]. As a result, an increase in intracellular osmotic fluid is present. Taylor et al [242] has suggested that an increase in plasma proteins produce an imbalance across the vessel wall as these proteins move into the interstitial space and fluid is drawn out. Free radicals may also play a role in edema as they give rise to proteolytic enzymes when the microcirculation is compromised. Lipid peroxidation initiated by free radicals decreases the barrier function of cell membranes and may be associated with muscle fibre necrosis and enzyme release following damaging exercise [112]. The oxygen diffusion distance from the capillaries can be up to four times greater than normal with hyperbaric oxygen. This is mainly due to the larger pressure gradient between capillary and tissue PO_2, which increases the number of cells that can be oxygenated when

cellular oxygenation is limited by edema [61]. Therefore, hyperbaric oxygen may be beneficial in reducing edema by ensuring adequate cellular oxygenation to maintain cellular energy production, allowing the cell to fuel its ATP driven pumps and channels, thus enhancing the reabsorption of fluid from the extracellular space and decrease edema [83, 225]. Furthermore, secondary vasoconstriction by HBO would reduce blood inflow by 20% without decreasing oxygen delivery. This would also reduce edema by decreasing the intravascular hydrostatic pressure, and establishing a more favorable pressure gradient for fluid movement out of the interstitial space back into the capillaries [225].

In contrast to studies that have demonstrated a reduction in edema by hyperbaric oxygen therapy [3, 5, 72-74, 91], there is inconclusive evidence that HBO therapy reduces edema with soft tissue athletic injuries. [95]. Our study indicates that this modality of treatment was not effective in minimizing the edema formation associated with exercise-induced muscle injury. This finding is similar to that of Mekjavic et al [225] who suggested that edema in their study was not of sufficient magnitude to establish the increased diffusion distances and increased interstitial pressure, which might promote tissue hypoxia.

Blood Analysis:

Creatine Kinase, Malondialdehyde and Interleukin-6

Creatine Kinase (CK)

Creatine kinase has been studied extensively in relation to muscle damage due to strenuous exercise since this plasma enzyme is found exclusively in the skeletal and cardiac muscle [41, 77, 127, 153, 160, 162]. It has been reported that the extent of release of this plasma enzyme is delayed and is a direct consequence of muscle damage and exercise [41, 112, 160, 162]. Furthermore, the magnitude and duration of the increase in CK activity is affected by the type and intensity of activity as well as previous level of activity [115]. It has also been cited that CK has a high degree of variability and its response varies between

individuals [41, 77, 162]. For example, one study in which subjects performed an eccentric exercise showed increases in CK activity up to 30 000 m-units/ml, while other subjects showed increases of less than 500 m-units/ml [248].

It was hypothesized that hyperbaric oxygen would attenuate the response of creatine kinase over the course of treatment. More specifically, 4-hours post-exercise levels would increase from baseline, but this response would be dampened in contrast to the control group. Analysis of the data did not demonstrate significant findings between groups for treatment with hyperbaric oxygen over the course of one week. Our findings are consistent with those of previous studies indicating that the eccentric exercise protocol, which was used in our investigation, resulted in a quantifiable muscle injury [100, 153, 177, 179]. However the lack of significance across both groups suggest that HBO was not effective in treating muscle injury or DOMS.

In females, the level of CK ranges anywhere between 45-230 u/L, with exercise, trauma, surgery and other ailments increasing levels 10-15 times normal range values [249]. Furthermore, some individuals may have increases up to 50 times the upper limit of normal range but this response is seen in conditions such as muscular dystrophy [249]. Elevation in levels may be seen as early as 2-4 hours post exercise, with values returning to normal by 48 and 72 hours post-injury. For the purpose of the present study, blood was withdrawn from subjects 4 hours post-exercise, followed by subsequent testing for 3 days (24, 48 and 72 hours) to examine increases in serum CK activity levels.

A statistical analysis on the creatine kinase data was performed on two separate occasions, one with the entire subject pool and a second with the removal of one responder in the experimental group who appeared to have a CK value well beyond the normal limits (>2500 *u*L). This was done to ensure that this high variability introduced into the data by this subject did not mask statistical

significant findings in the sample. Both analyses demonstrated no significance between groups for treatment effects.

The serum creatine kinase response to the eccentric exercise protocol in the current investigation showed considerable individual variability, although most subjects displayed an immediate increase in serum CK 4 hours post-exercise. Ebbeling and Clarkson [127] suggested that subjects who appear to be quite similar when performing the same amount of eccentric exercise can have changes in circulating CK activity that are different by orders of magnitude. Many factors influence intersubject variability, including age, gender, body composition and race [147]. In contrast, Ebbeling and Clarkson [127] point out that the increase in circulating CK is "unrelated to either the development of soreness, the amount of strength loss after exercise, fitness level of the subject, or lean body weight". Other investigators have looked at genetic variation and serum CK activity to understand the variability [112]. It is likely that the post-exercise rise in circulating CK activity is a manifestation of skeletal muscle damage but not a direct indicator [127].

Malondialdehyde (MDA)

A growing amount of evidence indicates that free radicals play an important role as mediators of skeletal muscle damage and inflammation after strenuous exercise [239, 240, 244]. It has been postulated that the generation of oxygen free radicals is increased during exercise as a result of mitochondrial oxygen consumption and electron transport flux, inducing lipid peroxidation [239]. Lipid peroxidation is potentially a very damaging process to the organized structure and function of membranes. Recent studies indicate that a) oxygen free-radicals mediate, at least in part, the increased microvascular permeability produced by reoxygenation, and b) free radical scavengers can reduce skeletal muscle necrosis occurring after prolonged ischemia [244]. The literature supports the notion of the interrelationship between ischemic tissue and inflammatory cells and therefore, concludes that capillary plugging by granulocytes and oxygen free

radical formation may contribute to the ischemic injury [244]. The role of reactive oxygen species in the mediation of exercise-induced oxidative damage to muscle and the protection offered by anti-oxidant defense systems have been have also been well studied [138, 139]. Malondialdehyde, a product of lipid peroxidation is a way of estimating free radical generation as a result of skeletal muscle damage.

It was hypothesized that the eccentric exercise protocol would induce skeletal muscle damage, thus ultimately giving rise to free radicals and lipid peroxidation. Hyperbaric oxygen therapy would have an effect on lipid peroxidation by enhancing the antioxidative defense mechanisms and increasing the biochemical defense mechanisms against free radicals [193]. As a result, HBO therapy would attenuate MDA levels.

Analysis of the data did not demonstrate significant findings for treatment with hyperbaric oxygen. Hyperbaric oxygen had no effect on reducing free radical damage and limiting lipid peroxidation levels as evidenced by malondialdehyde. The results of both groups demonstrated very little fluctuation in malondialdehyde levels, increasing slightly after the eccentric exercise protocol and decreasing close to baseline levels by day 5. The mean values for MDA in this study ranged between 4.0 and 4.6 nmol/ml. Other studies have reported MDA values in the range of 1.0 – 3.0 nmol/ml [229, 250, 251]. This difference may be due to the intensity and duration of the prescribed exercise protocol, the variability in sampling times for blood analysis and nature of injury. Child et al [251] only sampled MDA levels pre and immediately post-exercise. This, therefore would not allow us to determine what the levels of MDA would be at 24, 48 and 72 hours post-exercise. Furthermore, Novelli et al [250] examined biopsies taken from the right femoral quadricep muscle at three time points during aortic surgery.

One plausible explanation for not demonstrating significance in the present study could be that the eccentric exercise protocol didn't elicit enough skeletal muscle damage to observe changes post-exercise. In other words, the effect was minimal which did not allow us to observe a noticeable change with HBO treatments, as expected. If the exercise protocol induced sufficient muscle damage or injury, the levels of MDA would have increased higher than what was observed. Furthermore, if HBO therapy was beneficial in reducing lipid peroxidation, the present study might then have been able to see a noticeable effect and obtain statistical significance.

Another factor that may have played a role in lipid peroxidation detection may be the mode of assay sampling (i.e. direct spectrophotometry). MDA assay is the most generally used test in the appreciation of the role of oxidative stress in injury and disease. MDA is one of several products formed during the radical induced decomposition or breakdown of endoperoxides during the last stages of the oxidation of polyunsaturated fatty acids. Most often, at high temperature and low pH, MDA readily participates in nucleophilic addition reaction with 2-thiobarbituric acid (TBA), generating a red, fluorescent 1:2 MDA:TBA adduct [191]. Because of this fact, and facile and sensitive methods to quantify MDA (as the free aldehyde or its TBA derivative), the "TBA test" is used as a routine test to detect and quantify lipid peroxidation in a wide array of sample types [191]. This reaction that occurs is very sensitive but its specificity, even with improvement of pre-analytical (sampling, preservatives), and analytical stages (fluorescence, HPLC) is still a matter of debate [243]. MDA itself participates in reactions with molecules other than TBA and is a catabolic substrate. Only certain lipid peroxidation products generate MDA (invariably with low yields) and MDA is neither the sole end product of fatty peroxide formation and decomposition nor a substance generated exclusively through lipid peroxidation [191]. An extensive review of the literature has concluded that MDA determination and the TBA test can offer, at best, a narrow and somewhat empirical window on the complex process of lipid peroxidation [191, 234]. It is subject to interferences, which if not

considered, may lead to erroneous results. Future studies should perhaps focus on more sensitive and specific (chemical or physical methods) modes of lipid peroxidation assessment.

Interleukin 6 (IL-6)

Increasing numbers of reports have described the IL-6 response to injury [187-188, 231-235]. Eccentric exercise is associated with an increase in serum IL-6 concentrations and is significantly correlated with the concentration of CK in subsequent days following injury. The time course of cytokine production, the close association with muscle damage and the finding of IL-6 in skeletal muscle biopsies after intense exercise lend support to the idea that during eccentric exercise myofibers are mechanically damaged and that this process stimulates the local production of inflammatory cytokines [197]. IL-6 is an integral cytokine mediator of the acute phase response to injury and infection. It plays an active role in the post-injury immune response, making it an attractive therapeutic target in attempts to control hyperinflammatory-provoked injury [235].

It was hypothesized that the eccentric exercise protocol would induce an inflammatory response that would lead to the production of IL-6. This increase in plasma levels of IL-6 would be alleviated by hyperbaric oxygen treatments over the four days of therapy and the inflammatory response would be reduced by treatment.

Statistical analysis of the data demonstrated non-significant findings for both groups. This must be interpreted cautiously, as the sample size was quite small and standard errors of the means large. A great degree of variability in the analysis was demonstrated. This variability was unexplainable and may largely be attributed to error in laboratory technique. Another limitation we encountered in the study was that the laboratory, in conducting the analysis, contaminated and destroyed 8 of the 16 subjects (50%), thus leaving a total of four subjects per group (n=4). This therefore diminished our sample size and as a result, was not

sufficient enough attain significance to substantiate or refute the involvement of hyperbaric oxygen therapy in reducing IL-6 elevations during muscle damage and injury.

Examination of the means of both groups provided perplexing results. The experimental group demonstrated a relatively stable level of IL-6 while the control group showed an irregular pattern [Figure 11]. Overall, all subjects in both groups had variability in the response of IL-6 to the eccentric exercise protocol and subsequent treatment sessions. These results do not typify the normal course of IL-6 that has been reported in the literature. Biffl et al [235] has suggested that IL-6 concentration rose within 2-4 hours post-trauma. This was not evident in our investigation as IL-6 levels elevated 24 hours post-exercise for both groups. The magnitude of elevation of IL-6 is related directly to the degree of tissue injury [235], ranging <50 pg/ml in healthy individuals and rising as high as 1000 ng/ml in certain severe disease states [233]. Assuming that the results were reliable and valid, it appears that hyperbaric oxygen was doing something to the plasma levels of IL-6 in the experimental group, making the responses stable and less sporadic than the control group. This was evident in the graphical depictions of the means of both control and experimental groups [Figure 11]. Could administering 100% oxygen at high pressure have some impact on IL-6? A study by Rohde et al [188] at the Copenhagen Muscle Research Centre demonstrated that an increase of 570% in IL-6 concentration was seen in the control trial (pre-exercise to 2 hours post-exercise) and returned to pre-exercise levels at day 2. Bauer et al [233] reported that elevated levels of IL-6 could be found as early as a few hours or up to a few days and these levels fluctuate depending on the acute inflammatory response. For example, patients undergoing elective surgery reached IL-6 plasma concentrations of 100 pg/ml, while patients with viral meningitis ranged between 10 – 1000 ng/ml [233]. A review of the literature proved unsuccessful, as we were unable to support or explain the variable findings found in this study. Future studies need to further examine this question more in detail by perhaps focussing on the inflammatory response and examining

in more detail how hyperbaric oxygen therapy affects the ensuing cytokine response.

Magnetic Resonance Imaging

Magnetic resonance imaging, being a powerful non-invasive measurement, allowed us to determine whether the eccentric protocol did in fact produce exercise-induced muscle damage and as well as quantify this level of muscle injury as evidenced by muscle edema. Skeletal muscle T2 relaxation time has been associated with changes in the fluid component of injured muscle and used as a marker of edema, inflammation and injury [177, 179, 246]. This is consistent with previous findings that suggest that edema results from high intensity eccentric exercise [174, 177-179].

In the current study, it was hypothesized that hyperbaric oxygen treatment would decrease muscle edema that was induced by the eccentric protocol over the time course of therapy. Statistical analysis for both T2-weighted images and STIR images demonstrated non-significant findings for treatment effect over time. This was evident in all three muscles: rectus femoris, vastus medius, and vastus lateralis. Although T2 and STIR images indicated an increase in edema 24-hours post-exercise for the same three muscles, respectively, treatment with hyperbaric oxygen did not induce an effect sufficient enough to reduce edema and achieve statistical significance.

The nuclear magnetic resonance (NMR) signal from which MR images are constructed arises from the magnetic behavior of the hydrogen nuclei in tissue water and fat molecules when tissue is placed in a strong magnetic field. Inside a strong magnet, the hydrogen nuclei can be excited by the input of energy, specifically, by a "pulse" of energy at the resonant radiofrequency [226]. This excitation causes a fraction of the nuclei to oscillate together in the magnetic field in an orientation that generates a detectable magnetic signal that can be recorded electronically and as a result, form images [226]. However, immediately

after excitation, nuclei in different magnetic environments begin to oscillate differently, the oscillation begins to breakdown and the observed signal decays away [226]. Decay of the NMR signal is referred to as transverse relaxation time (T2 relaxation time) and is the time constant that characterizes the exponential decay of the signal after the initial excitation [226]. T2 relaxation time, however does not suppress fat on the observed images. Short Tip Inversion Recovery (STIR) images allows one to sort out tissue as this form of imaging only shows fluid (bright signal) and suppresses fat [APPENDIX D].

MRI is the most sensitive non-invasive imaging method for detection and quantification of muscle edema. T2 weighted images and STIR images are particularly sensitive for detection of edema and increased water content in body tissue.

The MRI data in this study is based on signal intensity measurements. MRI signal intensity measurements are expressed in arbitrary units, which were variable due to a number of factors. The signal intensity obtained from a specific volume of tissue depends on the physical properties of the tissue (such as water content, tissue composition, and tissue density), the spatial location of the tissue relative to the signal reception coil, the performance characteristics of the radiofrequency system, the performance characteristics of the reception coil, and the performance characteristics of the amplification system. Many of these characteristics are variable from moment to moment and from location to location within the body. To overcome this variability, this study used a ratio of signal intensity in the stressed muscle divided by the signal intensity simultaneously obtained in the corresponding exact anatomic location in the opposite quadricep muscle. This type of internal control overcomes variability associated with (for instance) body hydration, body position relative to the coil, and the operating characteristics of the MRI system.

This measurement system is susceptible to signal intensity variability resulting from asymmetric positioning of the body within the MRI scanner, however care was taken to ensure that all subjects were positioned in a symmetric and midline fashion within the scanner system. Another potential source of error is injury (acute or chronic) in the dominant (control) leg, which could affect signal intensity measurements. The images were visually inspected and any area of abnormality/injury in the control leg was avoided. Due to the expense involved in conducting MRI's on all subjects, this study had to limit image scans to 3 per subject. As a result, baseline images were taken, followed by a scan 24 hours post-exercise and finally 72 hours post-exercise. Ideally, images taken throughout the entire week of the study and perhaps even 96 and 120 hours post-injury would have been beneficial to see a pattern of recovery. By only taking images at 0, 24 and 72-hour time points, introduces the opportunity for potentially missing what is happening between those periods. Finally, enough muscle injury may not have been demonstrated by the eccentric exercise protocol to attain statistical significance in treating this soft-tissue injury with hyperbaric oxygen treatments.

CHAPTER 6:
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

Hyperbaric oxygen is a field of much controversy and skepticism. The handful of studies that have been published, differ in results as to whether this form of therapy proves effective in treating sport and exercise-related injuries. The purpose of this study was to determine whether intermittent exposure to hyperbaric oxygen played a beneficial role in the recovery from an acute soft tissue injury. A group of sedentary female students (n=16) were randomly assigned to one of two groups (control [*air*] & experimental [*hyperbaric oxygen*]). The subjects performed a high intensity exercise protocol, followed by the administration of four daily treatment sessions. No significant differences ($p<0.05$) among any of the dependent variables were observed between those treated with hyperbaric oxygen and those that received normoxic conditions.

Conclusions

From a review and analysis of pain, strength, quadricep circumference, CK, IL-6, MDA and MRI data, it appears that hyperbaric oxygen treatment did not have any effect on the following:

1. reducing perceived muscle soreness over the five-day testing period.
2. improving eccentric muscle strength after the exercise protocol over the four-day course of treatment.
3. reducing edema as evidenced by 10 and 20 cm quadriceps circumference measurements.
4. reducing the CK response, which is exacerbated by skeletal muscle damage.
5. reducing IL-6 levels which have recently been demonstrated to increase considerably following eccentric exercise.

6. reduce MDA levels indicative of lipid peroxidation, which occurs during eccentric exercise and subsequent skeletal muscle damage.
7. reducing edema as evidenced by MRI imaging

Recommendations for future studies

The present study does not lend support to the efficacy of using this treatment modality in soft-tissue injuries. However, a more focused study on fewer "key" variables and a larger sample size may add light to this area of research. The cost of doing the above study was quite expensive and therefore, modifications were implemented to reduce costs (e.g. 5 to 3 MRI scans/subject).

Future studies should:

1. focus on certain important variables such as strength, blood parameters (i.e. MDA & IL-6) and MRI over more time periods. These variables do not carry the subjective component that pain and quadriceps circumference measurements employ and the variability component that CK has been cited as having.
2. increase the sample size to ensure a higher power and reduction of type 2 error.
3. examine plasma PGE_2, since prostaglandins are produced by invading macrophage during injury and have been cited to sensitize pain receptors. Furthermore, PGE_2 has been implicated in the generation of inflammatory pain [156, 157].
4. examine whether the DOMS model is appropriate for inducing injury to the muscle. In other words, does this type of soft-tissue injury create an oxygen diffusion distance great enough for hyperbaric oxygen to demonstrate its beneficial effects, increasing the oxygen diffusion gradient. Investigations should perhaps focus on other types of injuries such as ligamentous injuries, where there is an increase in the oxygen diffusion

distance and the need for a reduction in the inflammatory process is warranted.

It is worth noting that the HBO treatment protocol that was used (60 minute sessions at 2.0 ATA, once a day for four days) in this study was based on previous research protocols looking at the therapeutic effects of this modality. However, pressure, duration of treatment, frequency and total number of treatments vary for a given indication. Care must be taken to ensure that oxygen toxicity levels are closely monitored. Four treatment sessions were chosen from a practical perspective, as an athlete being treated for an injury should have indication of significant improvement in the rate of recovery within several days.

Caution should be applied when negating the effects of hyperbaric oxygen therapy on soft tissue injuries based on the results of this study. Limitations of the study should be carefully considered as many factors may affect the therapeutic effects of HBO on muscle injury. Harrison et al [246] suggest that HBO therapy will be effective in the treatment of athletic injuries that involve a greater magnitude of soft tissue damage or for injuries in which oxygen availability may be more of a limiting factor due to the location of the injury or magnitude of local edema. Furthermore, researchers are limited due to issues of practicality, expense of treatment, toxicity levels and time. Although this study shows inconclusive results with respect to hyperbaric oxygen therapy, good quality research still needs to be conducted in this area, thus adding to the knowledge base on this issue as to its beneficial or non-beneficial effects on muscle tissue damage and injury.

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APPENDIX

APPENDIX A: INFORMED CONSENT FORM

The Effects Of Intermittent Exposure To Hyperbaric Oxygen For The Treatment of an Acute Soft Tissue Injury

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Shelina Babul, BSc, PhD Student
University of British Columbia, 908-9154
Email: sbabul@home.com

Background:
I understand that I am being asked to participate in this study because I am presently interested in volunteering for the study, which will be spread through a period of 1 week. I have met all the inclusion and exclusion criteria for the study and am fully aware that exercise-induced muscle soreness will be induced and treated with the use of hyperbaric oxygen.

The experimental treatment will consist of five sessions of hyperbaric oxygen exposure at 100% at a compression level of 2 atmosphere absolute (ATA) [equivalence of 33 feet below sea level]) for a period of 60 minutes during each session. The standard control group protocol consists of 21% oxygen inspiration at 1.2 ATA, again for 4 sessions at 60 minutes per session.

Purpose:

The investigators are trying to determine whether exposure to hyperbaric oxygen will enhance the rate of recovery from acute soft tissue injury. Measurement of pain will be assessed by using the visual analog scale while strength/torque will be measured with the KinCom Dynamometer. Magnetic resonance imaging (MRI) [visual non-invasive images of the quadricep muscle] and blood levels will be analyzed to allow for inflammation and enzyme level assessment.

Study Procedure:

Should I be chosen to participate in this study, I will be asked to attend 4 sessions of treatment (1 per day for 5 days). In addition, I am fully aware that 12 ml of blood (5ml is equivalent to 1 tsp.) will be withdrawn prior to and 6 hours post-exercise, as well as each day following treatment and magnetic resonance imaging (MRI) will also be taken of my quadricep muscle. I will also be asked to refrain from any form of exercise 12 hours post-injury and keep a brief diary of my activities as a reference. I understand that I will be placed in either a control or experimental group by randomization of names using a computer.

In order to avoid bias, neither the subject nor the investigator will know what treatment the subject is receiving. However, in case of an emergency, the code can and will be broken.

Exclusion:

I understand that I may be excluded from the study if I play on a team sport, run or weight train as part of my physical regimen, more than 3 hours per week. Individuals whose activities involve jumping and/or squatting will also be excluded from the study. In addition, individuals who have experienced delayed-onset muscle soreness to their quadriceps in the last three months, who have had a past history of severe joint injury, arthritis or other chronic illnesses and who are taking prescription drugs or analgesics will be excluded. Hyperbaric oxygen contraindications (e.g. diabetes, lung cysts, epilepsy, upper respiratory tract infections, pregnancy, fever or confinement anxiety) will also be evaluated.

Risks and Benefits:

I understand that the risks are minimal in this study, with mild aural barotraumas (ear ache), nausea, tooth and sinus pain and blurred vision occurring rarely. The benefit, if successful, will be no pain and a return of strength of the quadricep muscle sooner than the normal course of recovery for this condition.

Confidentiality:

I understand that any information resulting from this study will be kept strictly confidential and all documents will be identified only by a code number and kept in a locked filing cabinet. I will not be identified by name in any report of the complete study.

Contact:

If I have any concerns about my treatment or rights as a subject, I may contact Dr. R.D. Spratley, Director of Research Services at the University of British Columbia, at 822-8595.

If I have any questions or desire further information with respect to the study, I should contact any of the above investigators.

New Findings:

I will be advised of any new information that becomes available that may affect my willingness to remain in this study.

Patient Consent:

I understand that participation in this study is entirely voluntary and that I may refuse to participate or withdraw from the study at any time without consequences.

I have received my copy of the consent form for my own records.

I consent to participate in this study.

Patient Signature	Date
Witness Signature	Date
Investigator's Signature	Date

APPENDIX B: VISUAL ANALOG SCALE

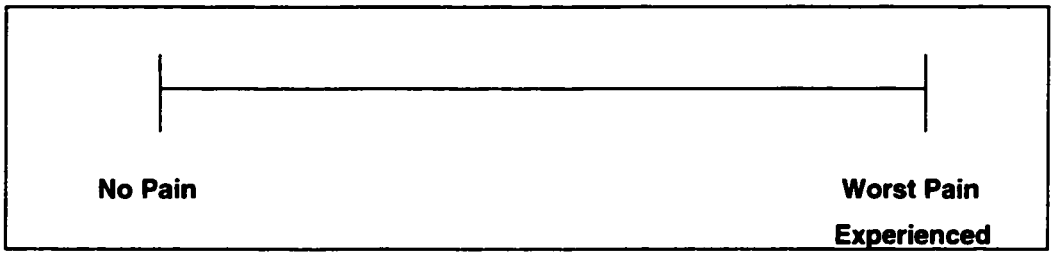
Subject #:

Date:

Time:

Treatment Day #:

Test # :



Location of pain: _____

_____

_____

GIRTH MEASUREMENT

Measurement #1:

10 cm measurement = _____

20 cm measurement = _____

Measurement #2:

10 cm measurement = _____

20 cm measurement = _____

HEIGHT = _____

WEIGHT = _____

Treatment times = _____

Time from DOMS to Treatment = _____

APPENDIX C: FIGURES A-Z (RAW DATA)

Figure A: Individual pain measurements over the 5 day testing period (control group).

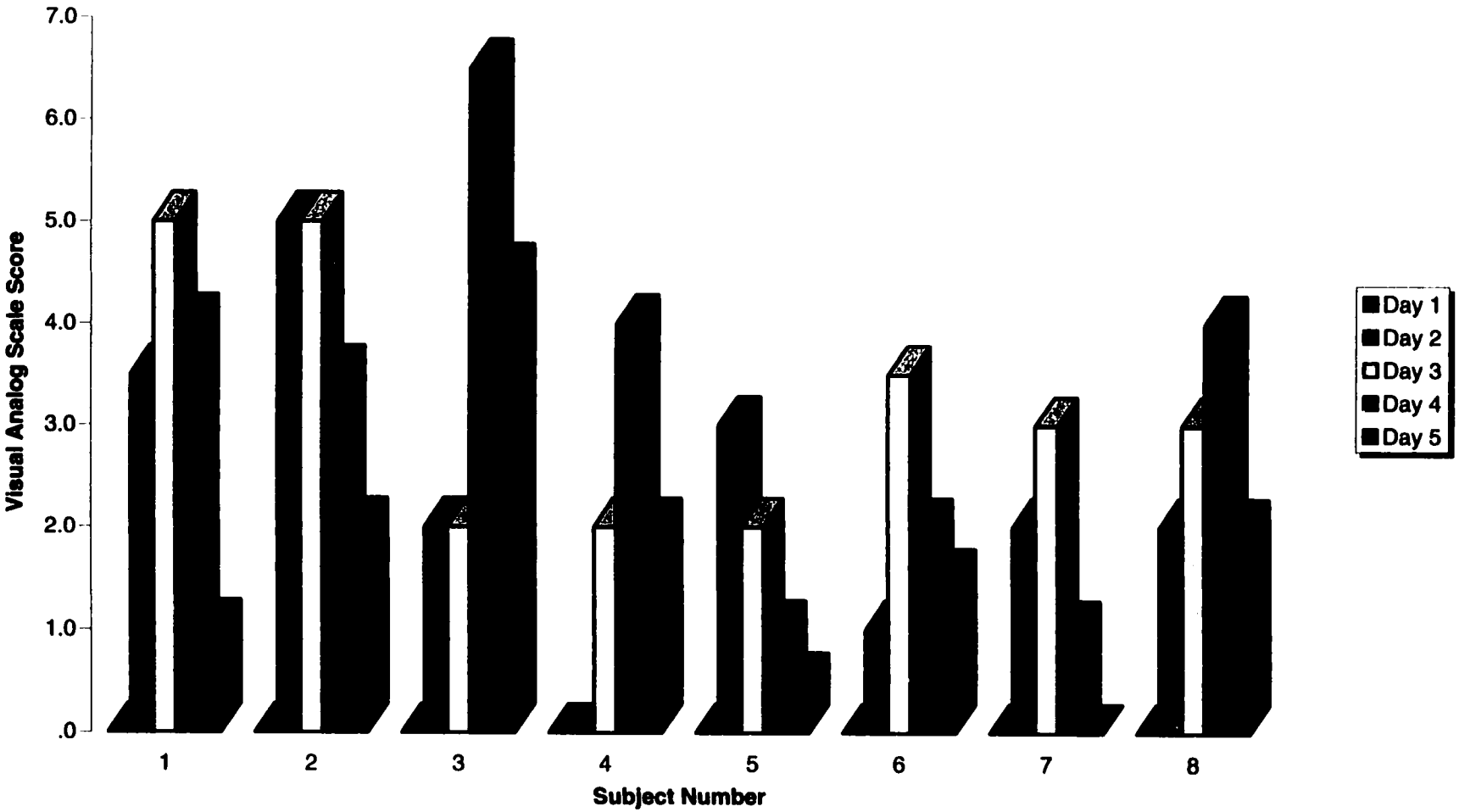


Figure B: Individual pain measurements over the 5 day testing period (experimental group).

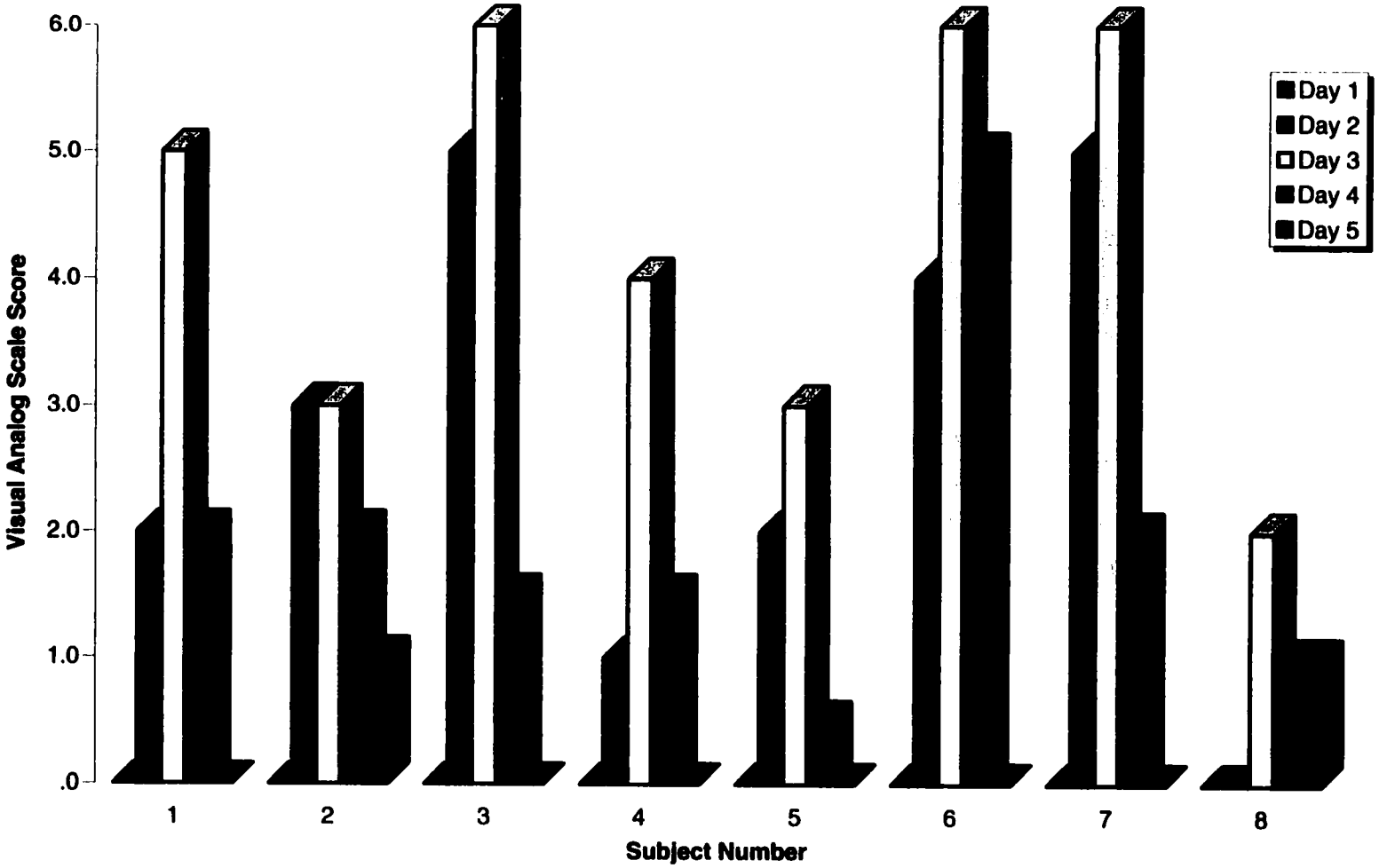


Figure C: Individual isokinetic eccentric torque over the 5 day testing period (control group).

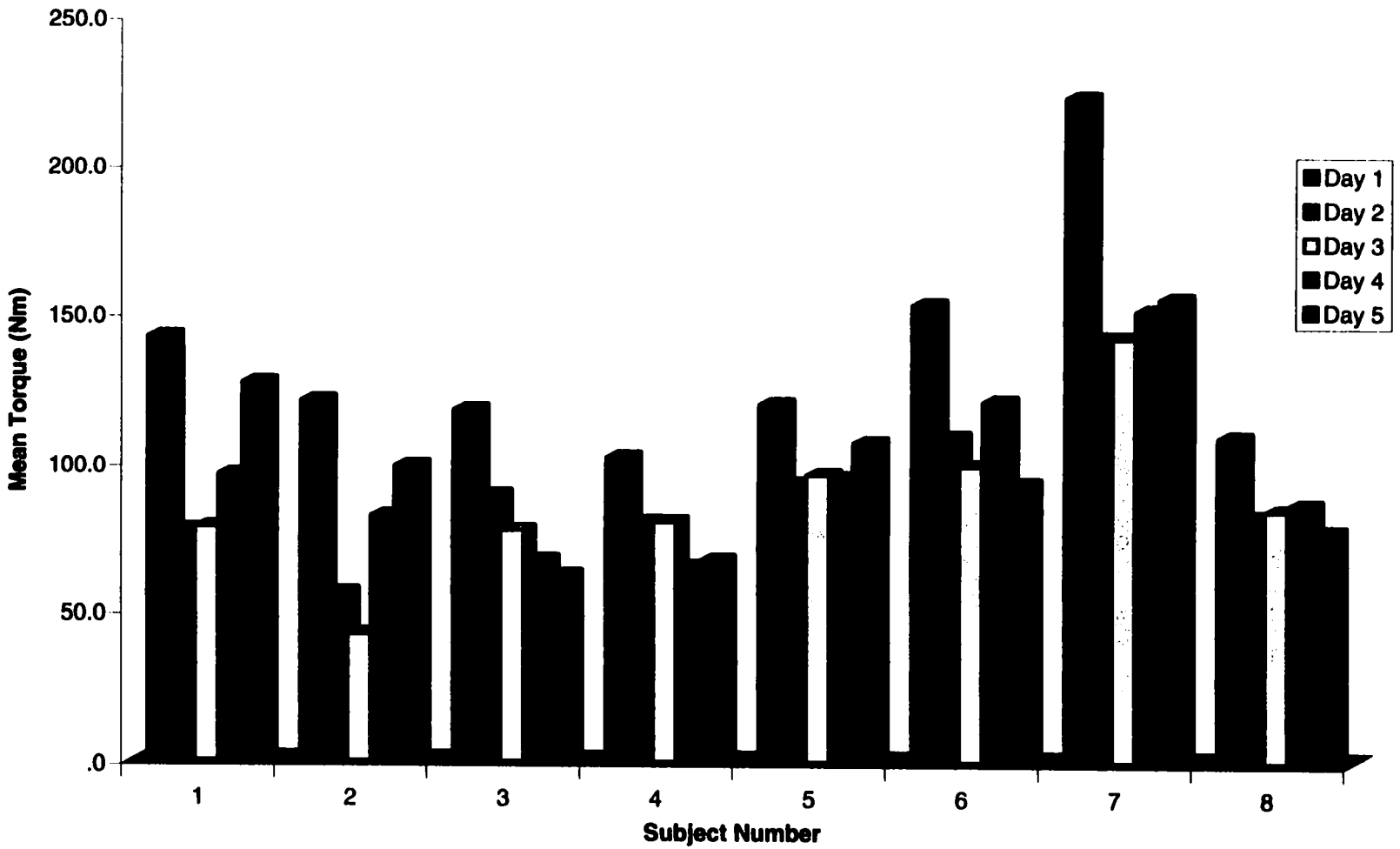


Figure D: Individual isokinetic eccentric torque over the 5 day testing period (experimental group).

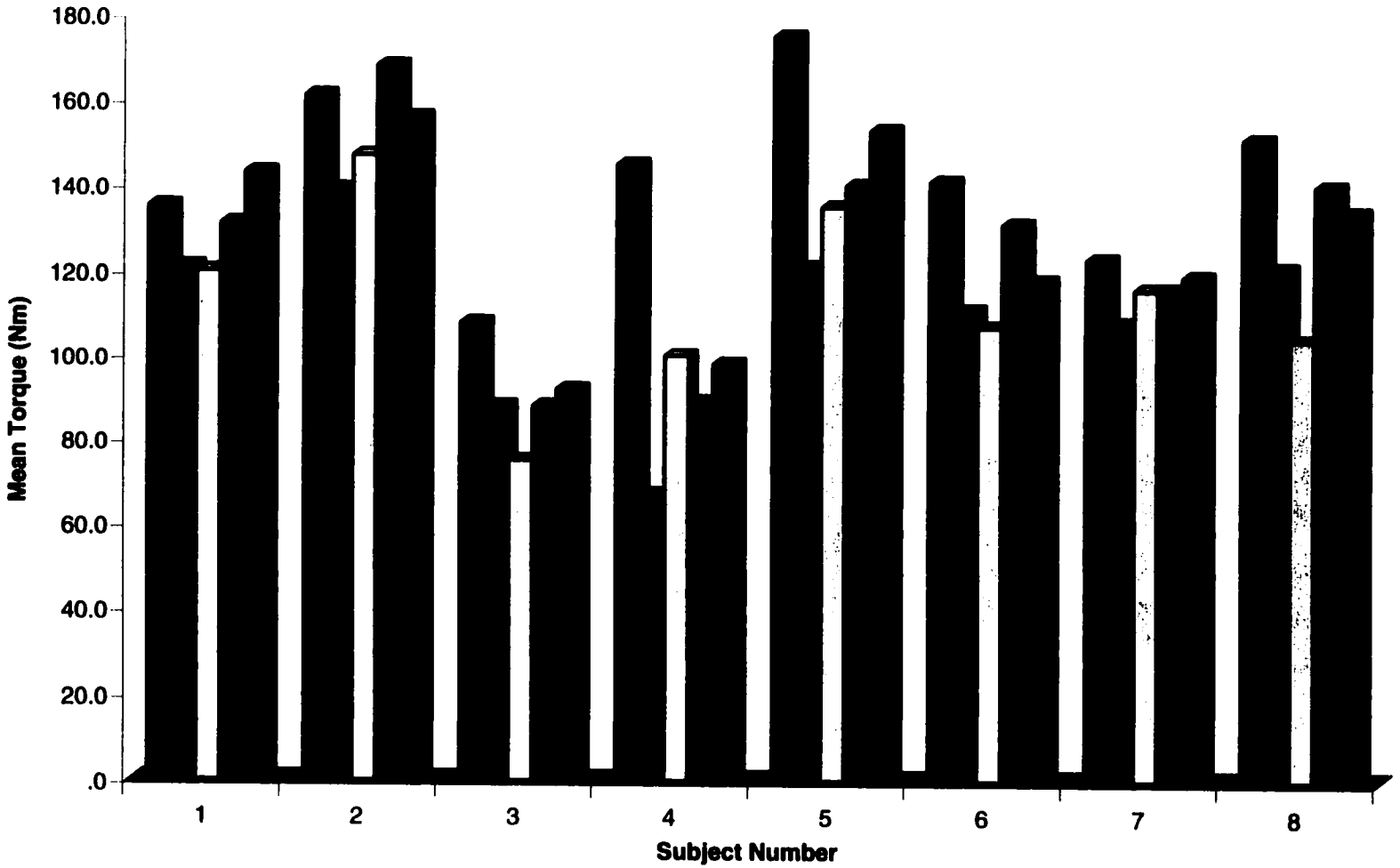


Figure E: Individual 10 cm quadricep circumference over the 5 day testing period (control group).

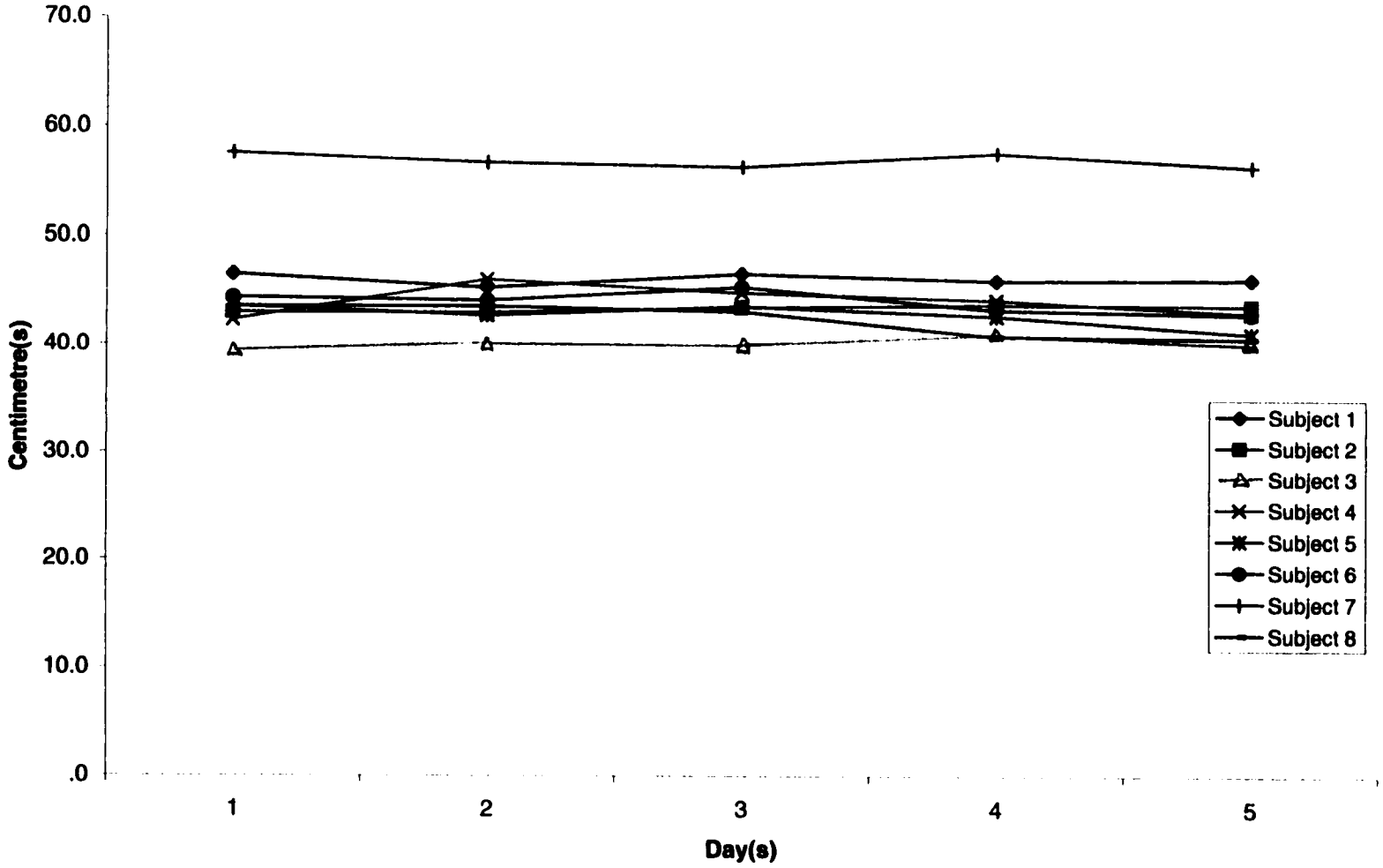


Figure F: Individual 10 cm quadricep circumference over the 5 day testing period (experimental group).

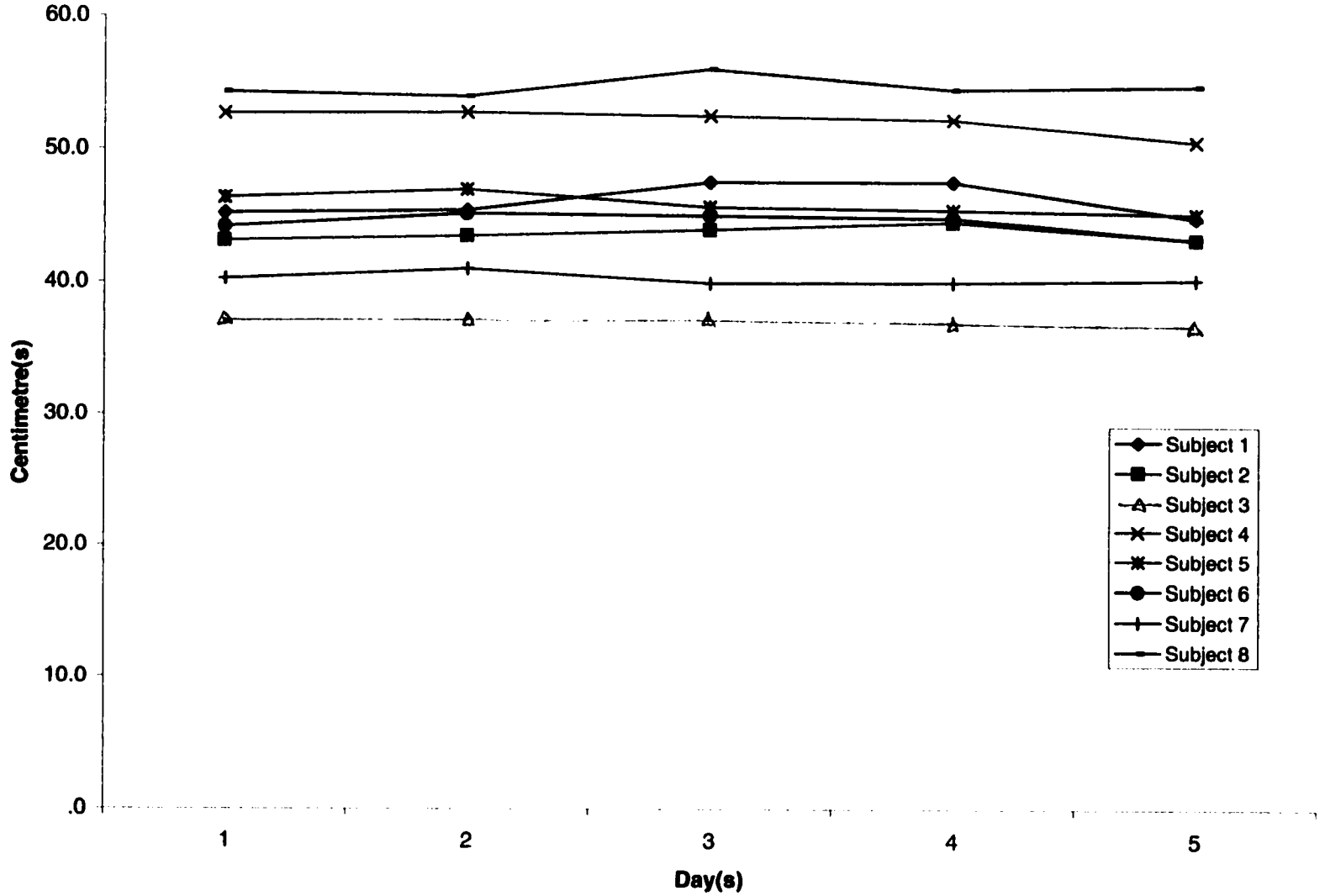


Figure G: Individual 20 cm quadricep circumference over the 5 day testing period (control group).

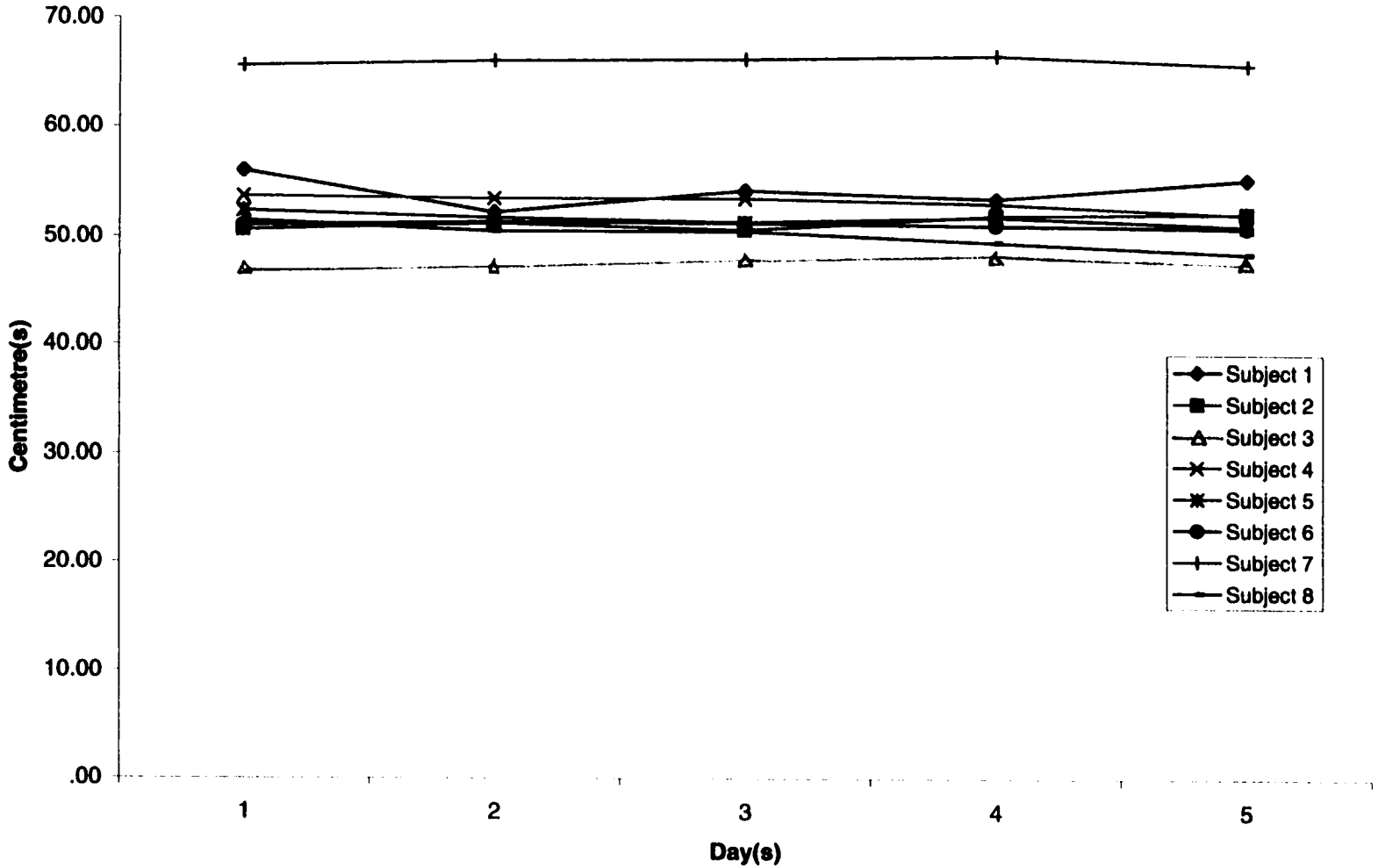


Figure H: Individual 20 cm quadricep circumference over the 5 day testing period (experimental group).

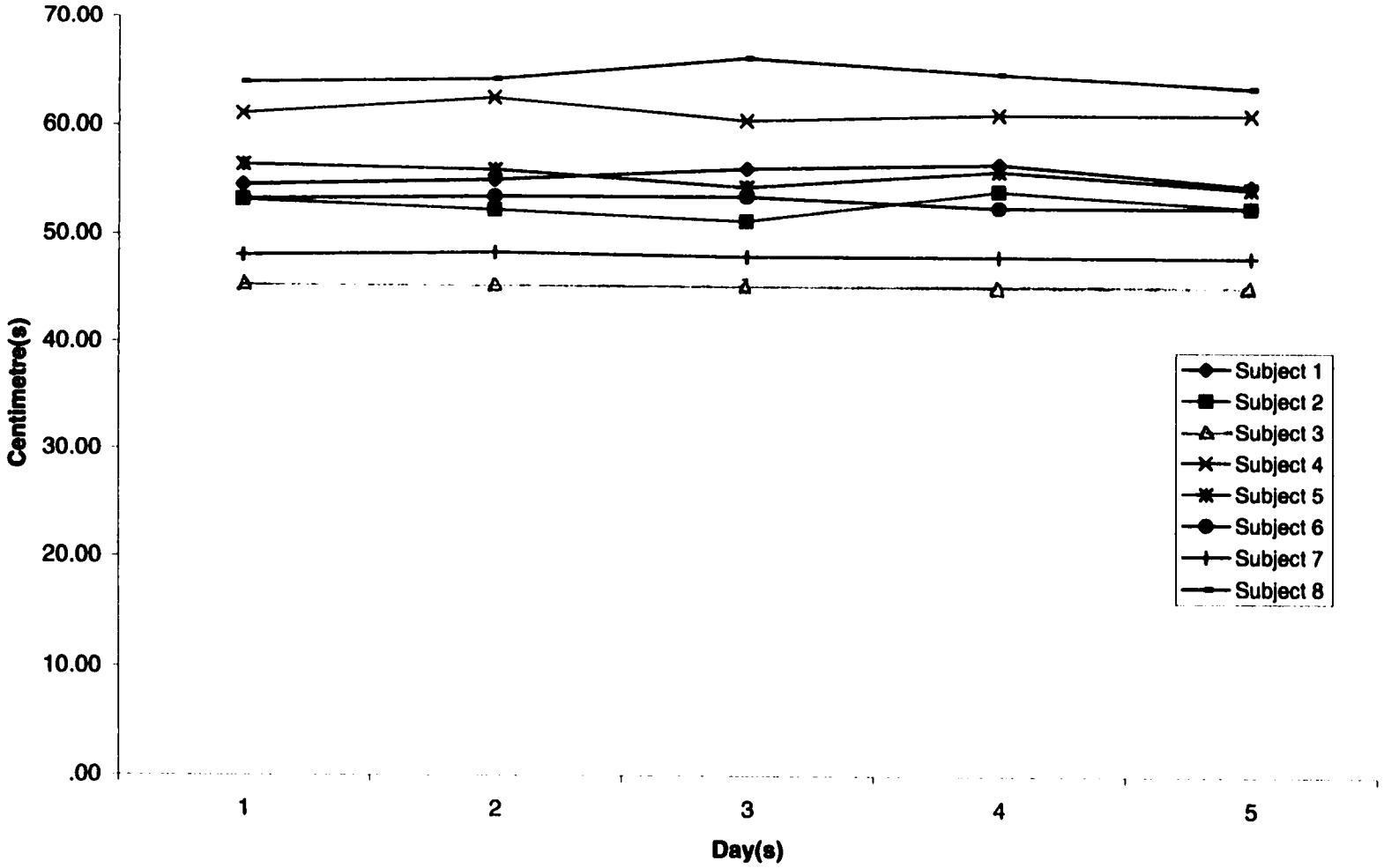


Figure I: Individual creatine kinase (CK) levels over the 5 day testing period (control group).

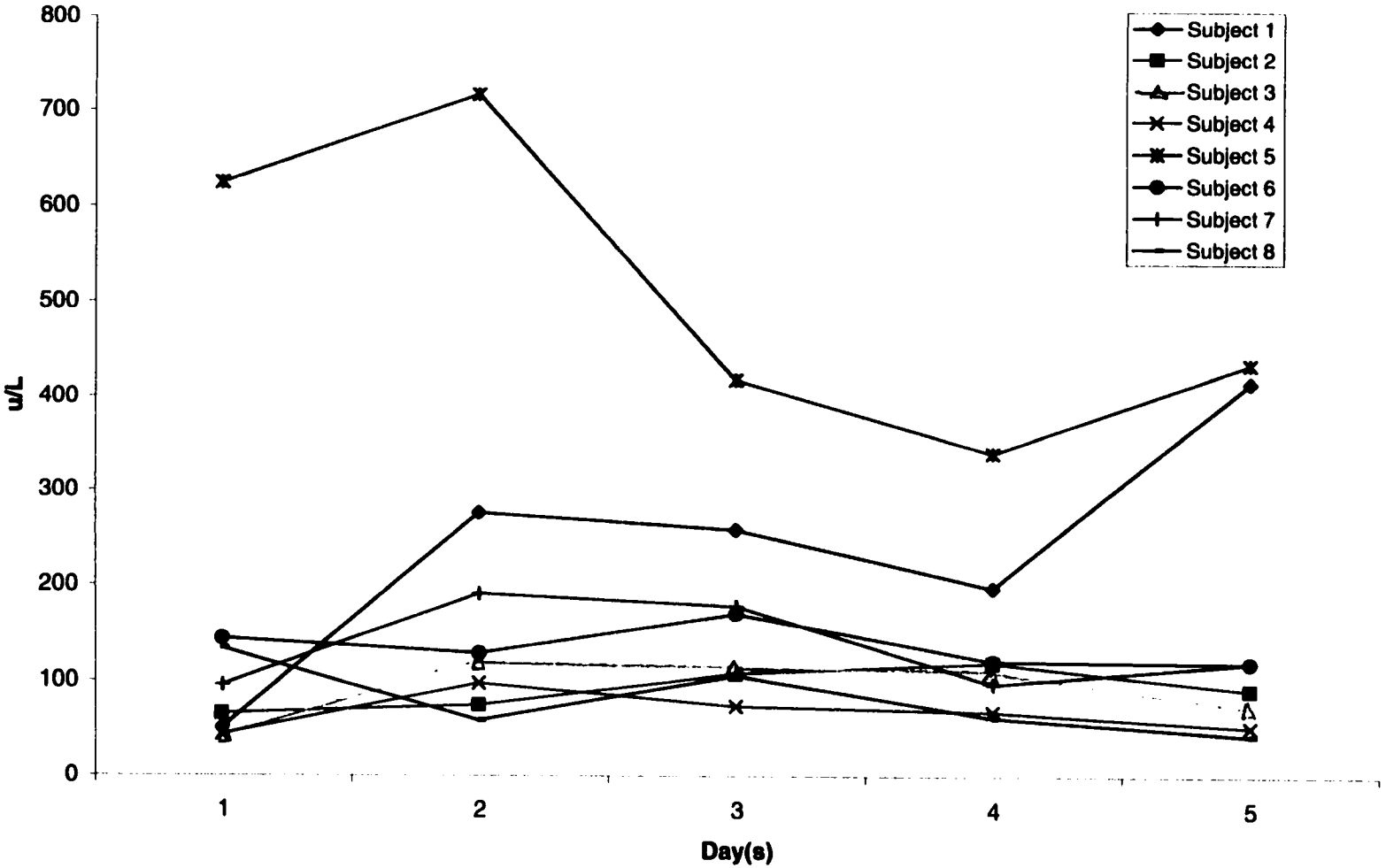


Figure J: Individual creatine kinase (CK) levels over the 5 day testing period (experimental group).

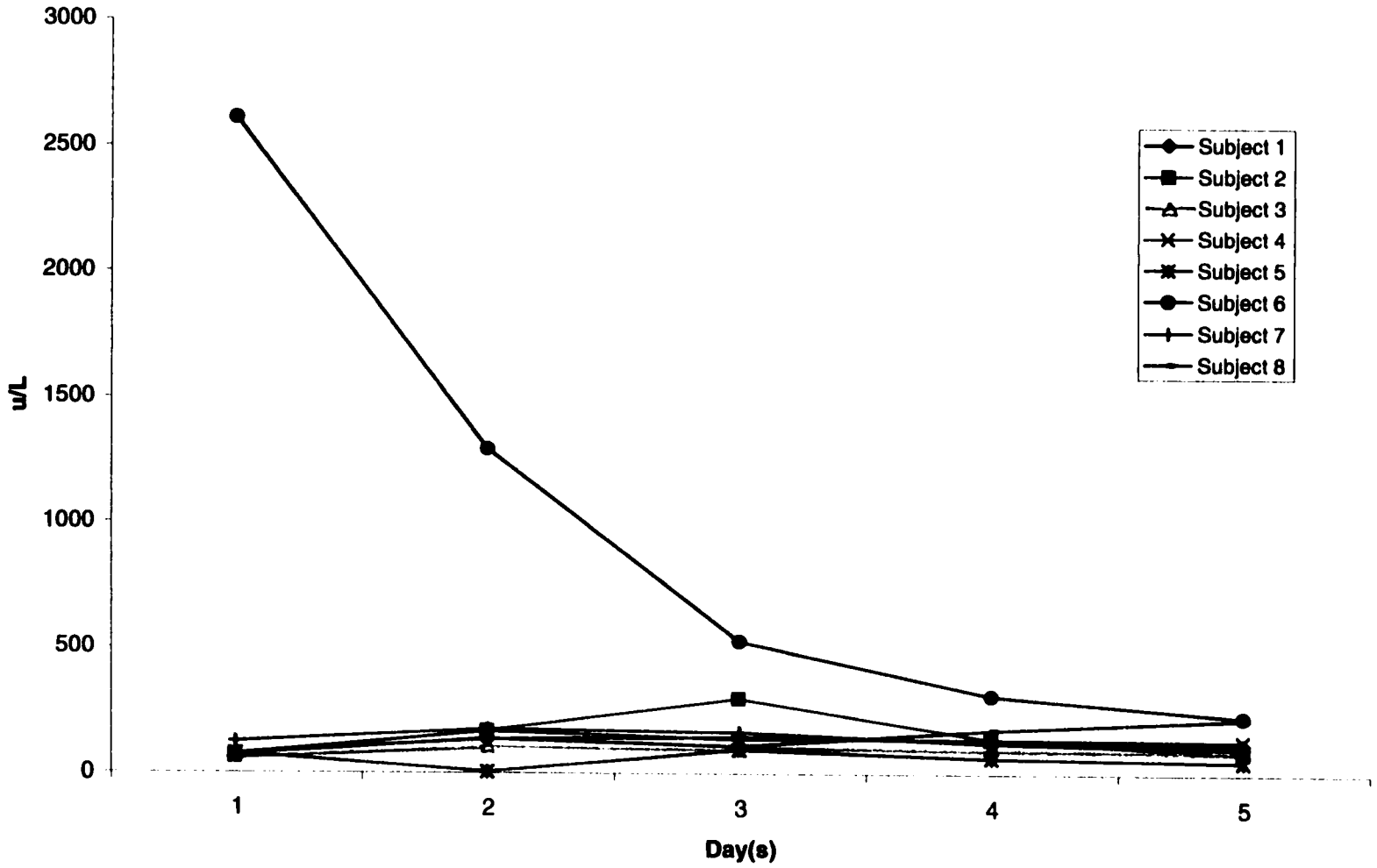


Figure K: Individual malondialdehyde (MDA) levels over the 5 day testing period (control group).

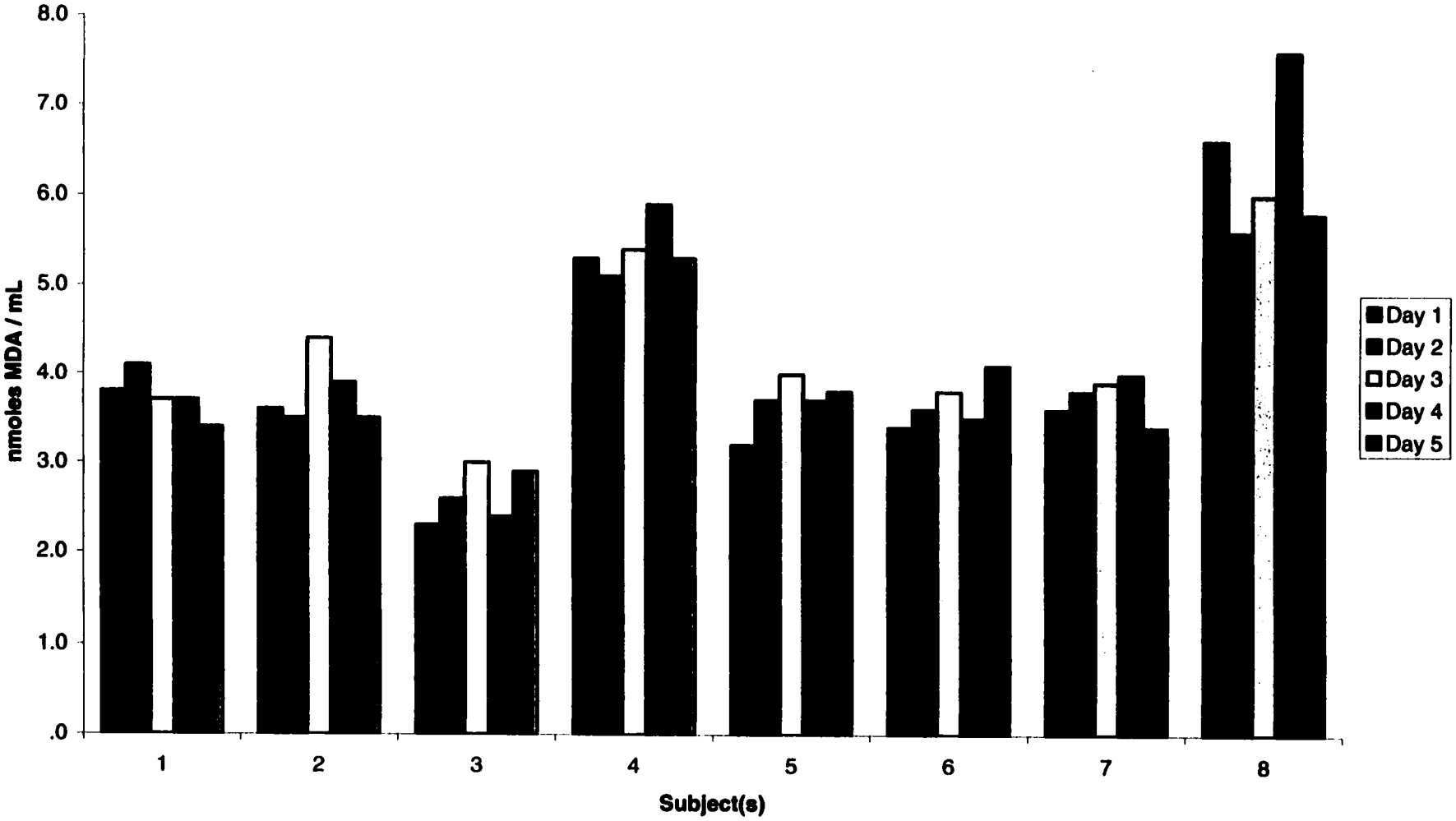


Figure L: Individual malondialdehyde (MDA) levels over the 5 day testing period (experimental group).

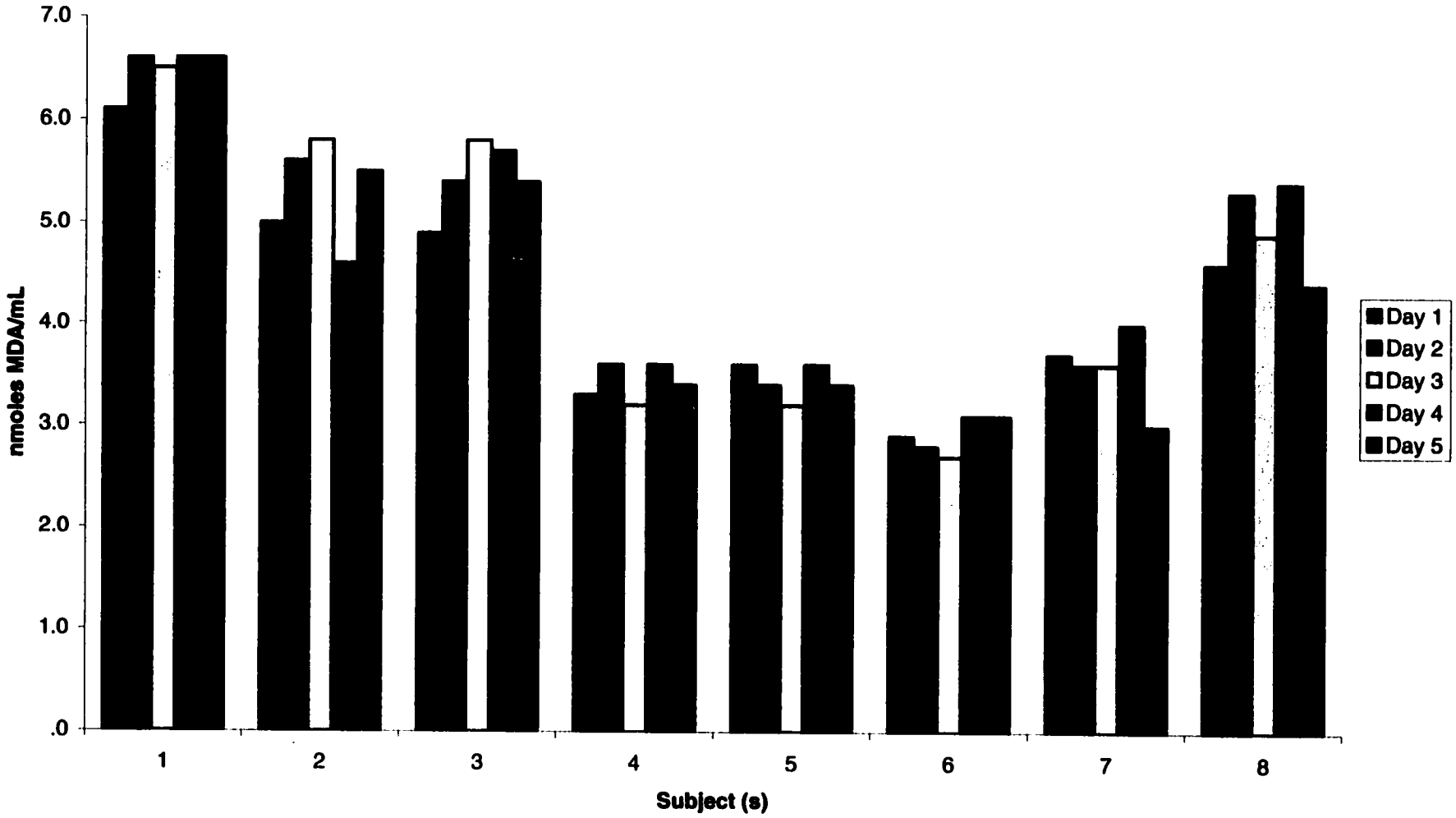


Figure M: Individual interleukin-6 (IL-6) levels over the 5 day testing period (control group).

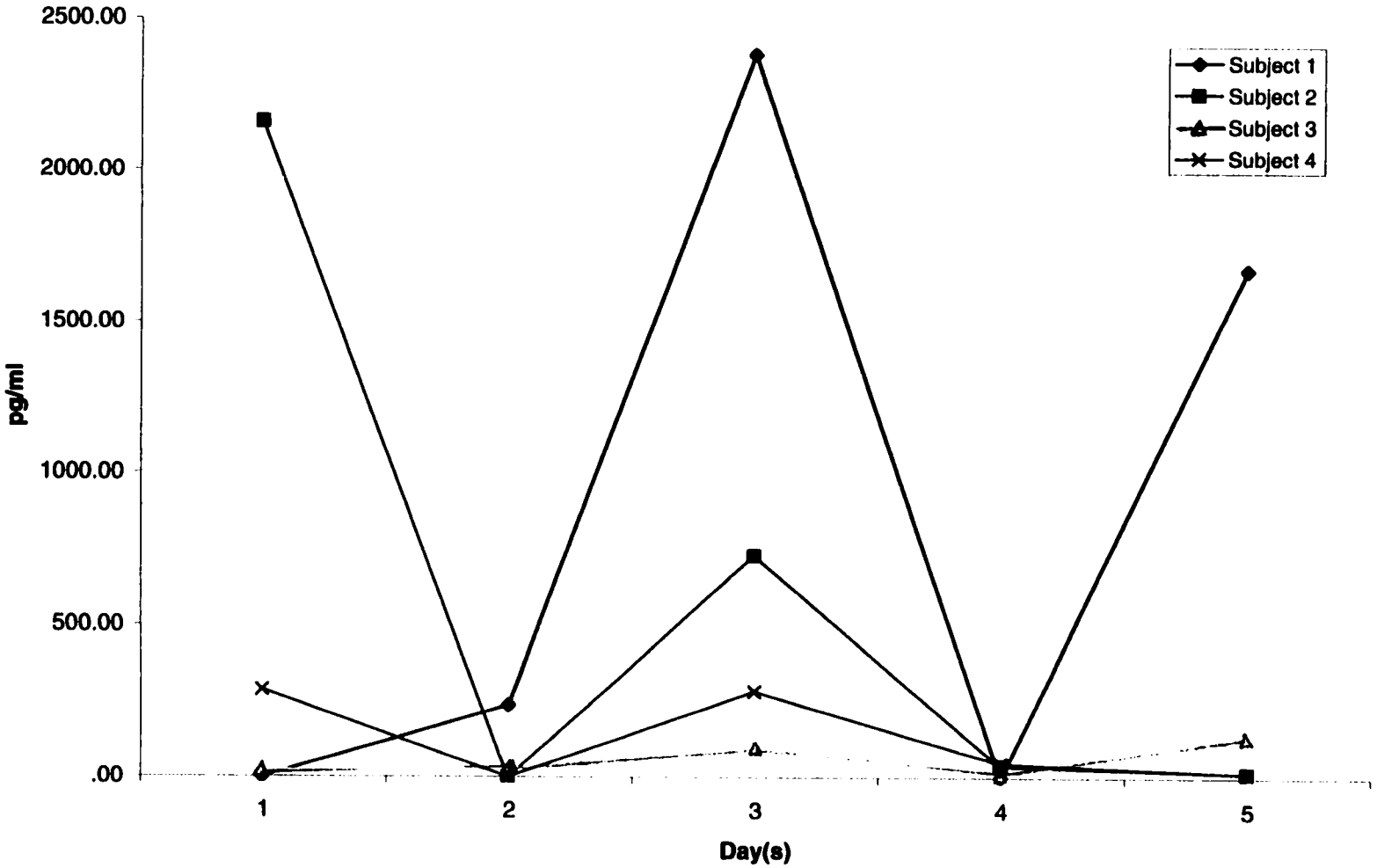


Figure N: Individual interleukin-6 (IL-6) levels over the 5 day testing period (experimental group).

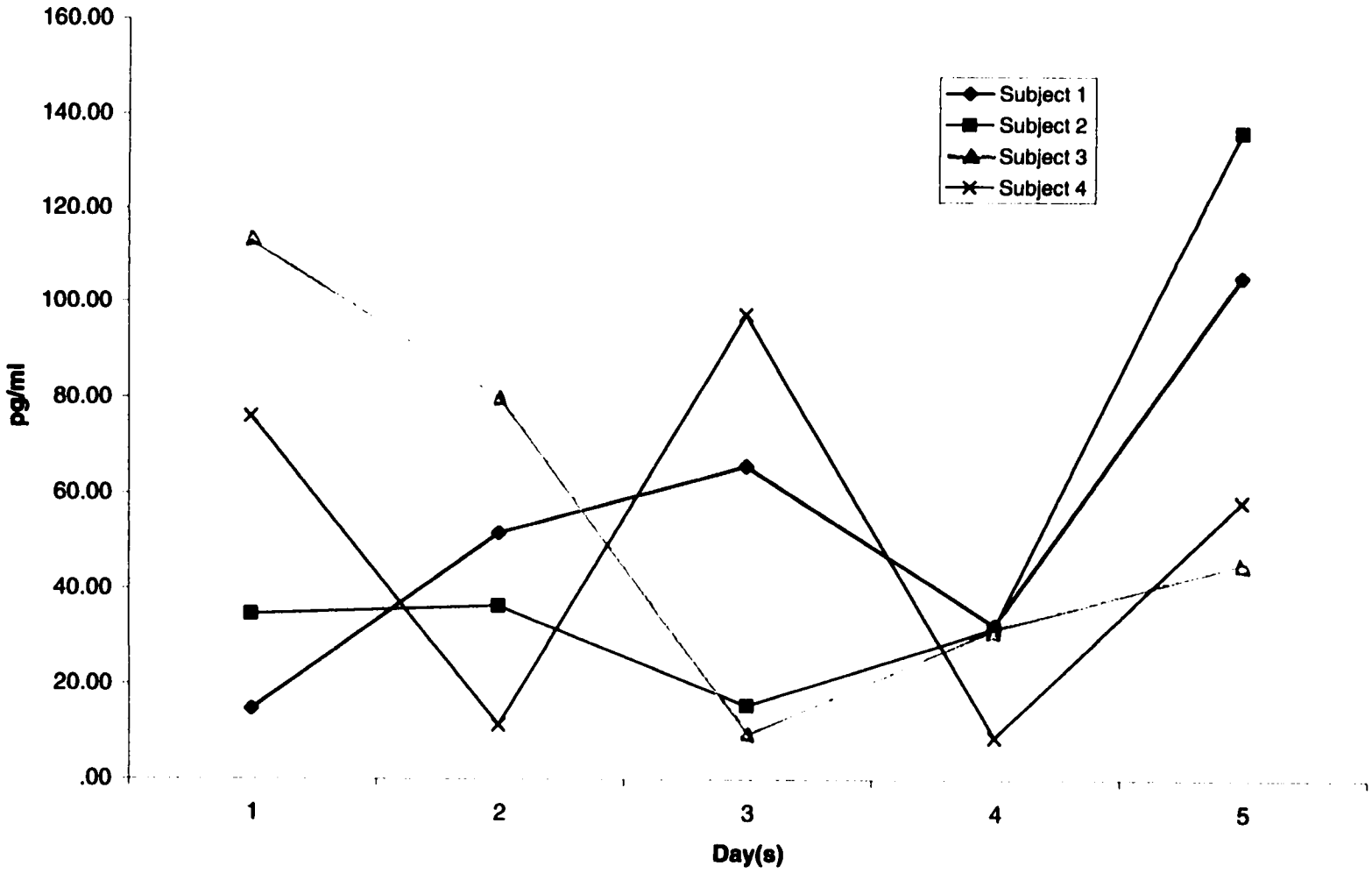


Figure O: Individual T2 relaxation times (msec) over the 5 day testing period for the rectus femoris muscle (control group).

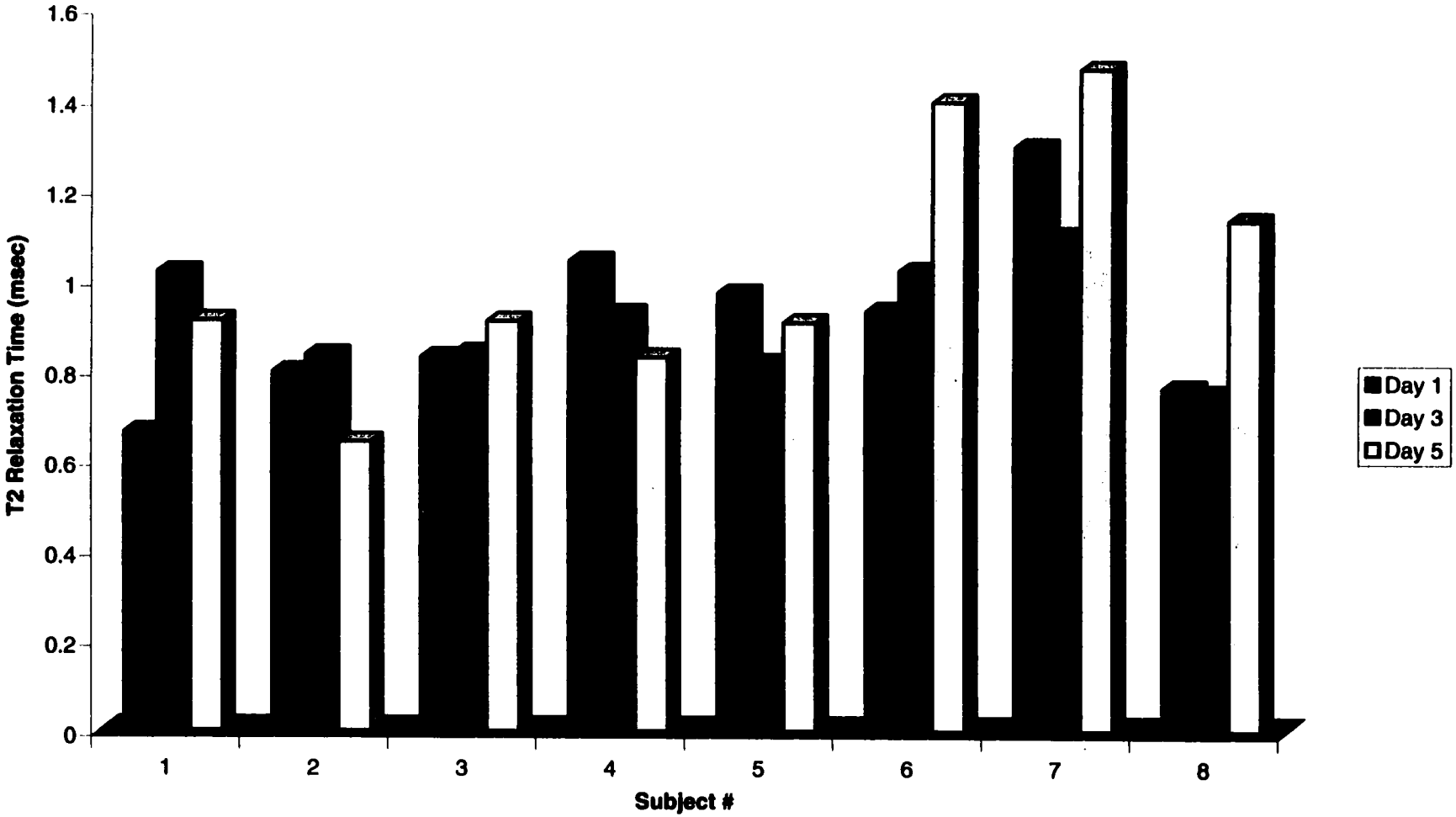


Figure P: Individual T2 relaxation times (msec) over the 5 day testing period for the rectus femoris muscle (experimental group).

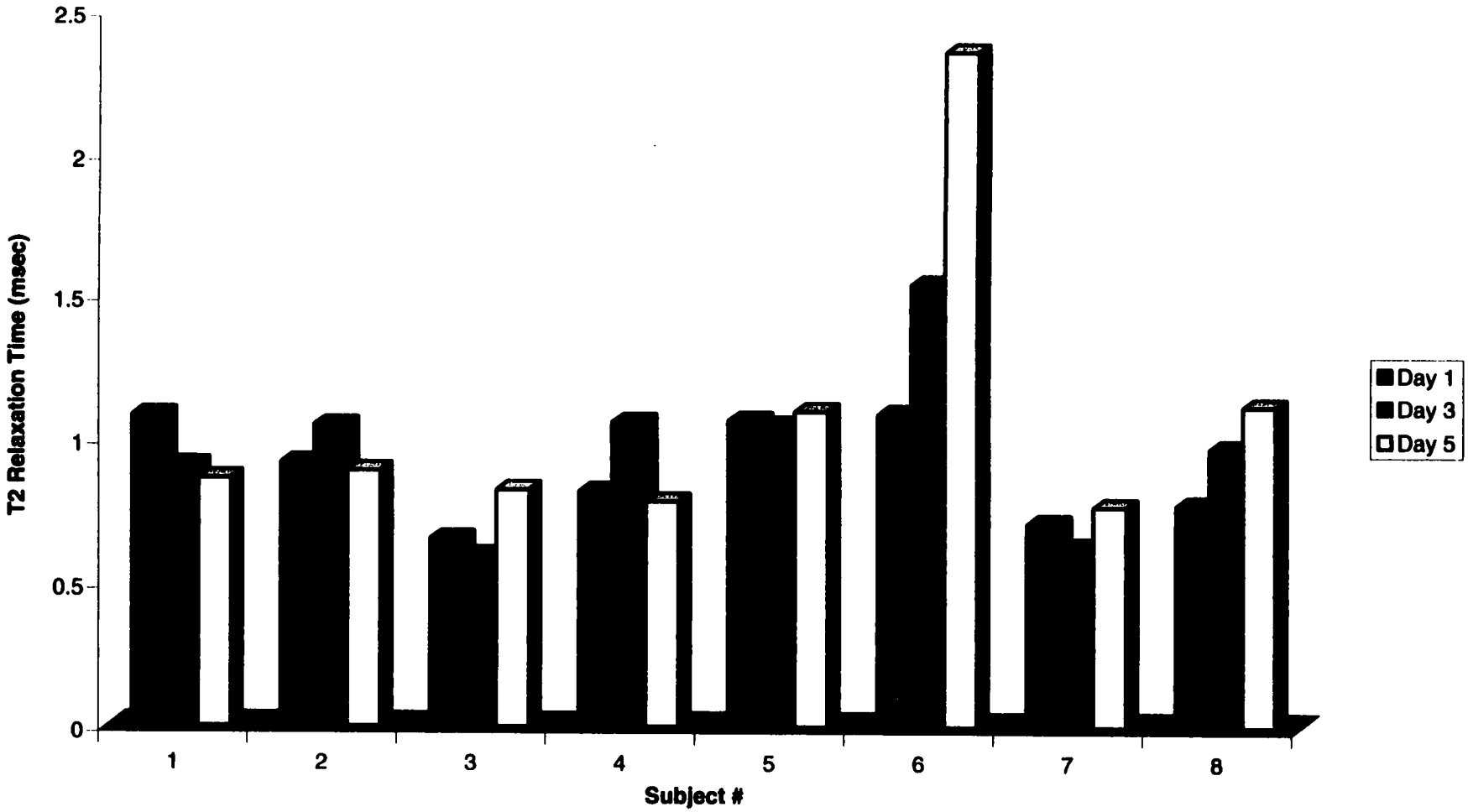


Figure Q: Individual T2 relaxation times (msec) over the 5 day testing period for the vastus intermedius muscle (control group).

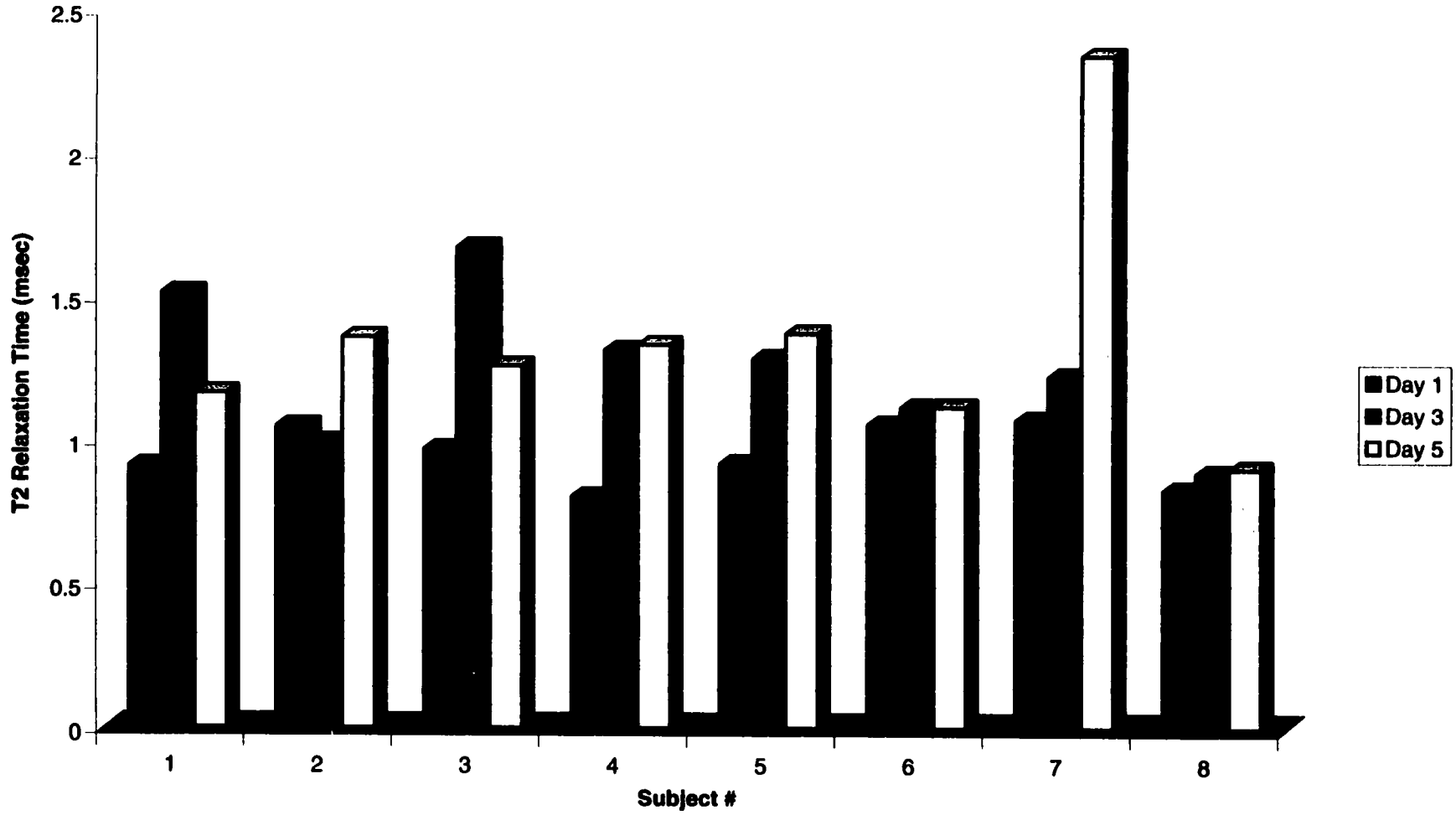


Figure R: Individual T2 relaxation times (msec) over the 5 day testing period for the vastus intermedius muscle (experimental group).

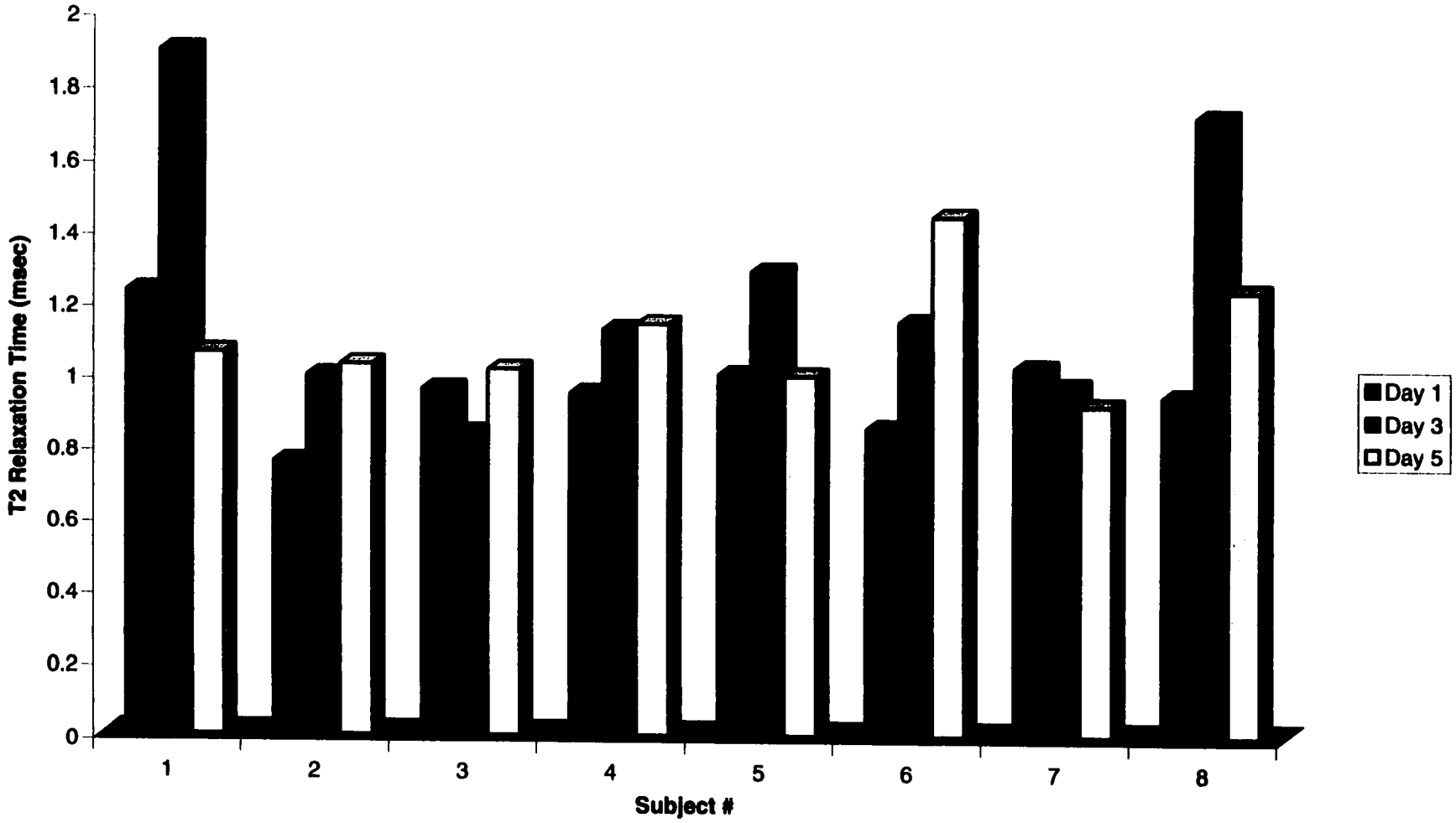


Figure S: Individual T2 relaxation times (msec) over the 5 day testing period for the vastus lateralis muscle (control group).

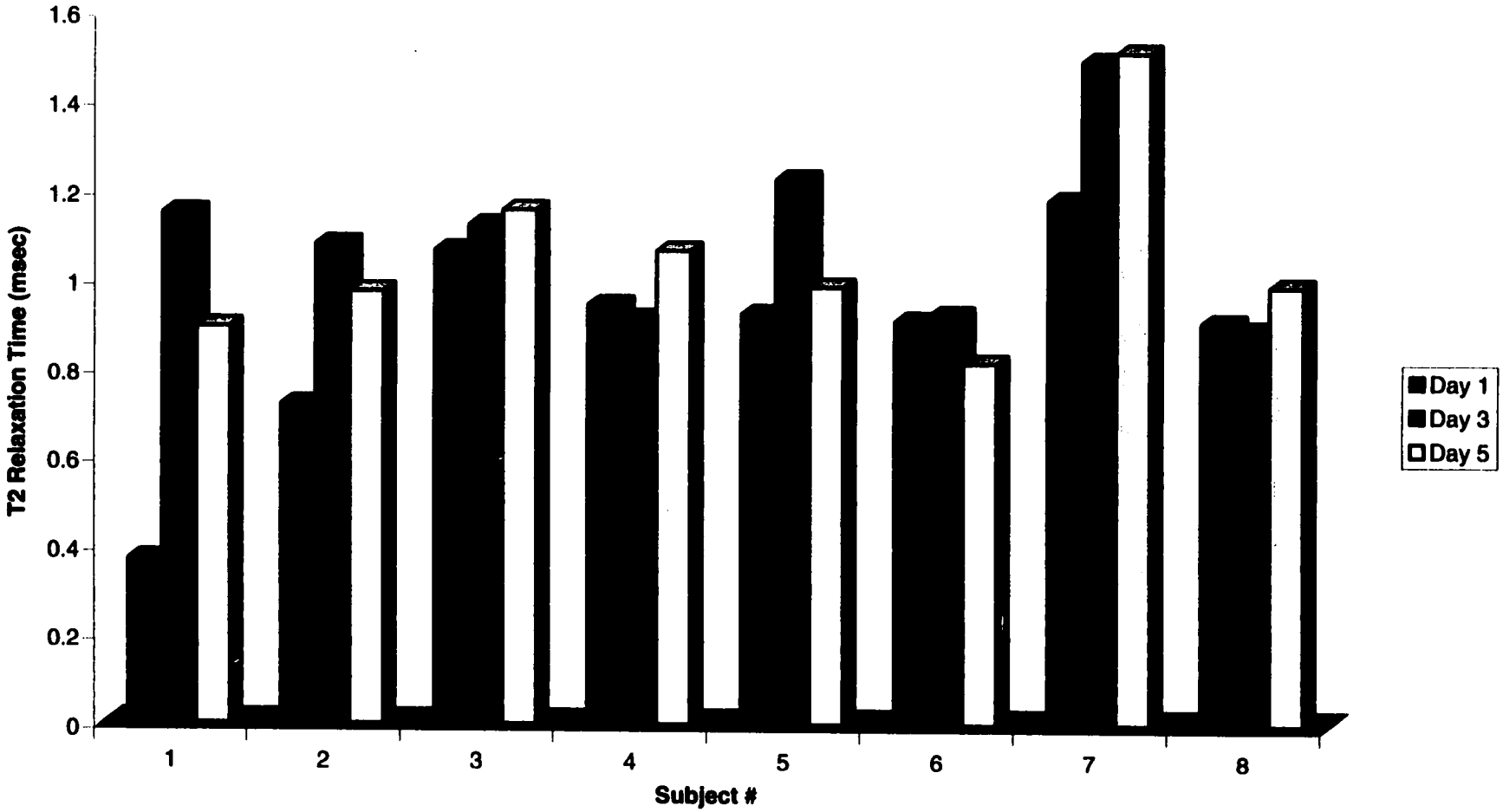


Figure T: Individual T2 relaxation times (msec) over the 5 day testing period for the vastus lateralis muscle (experimental group).

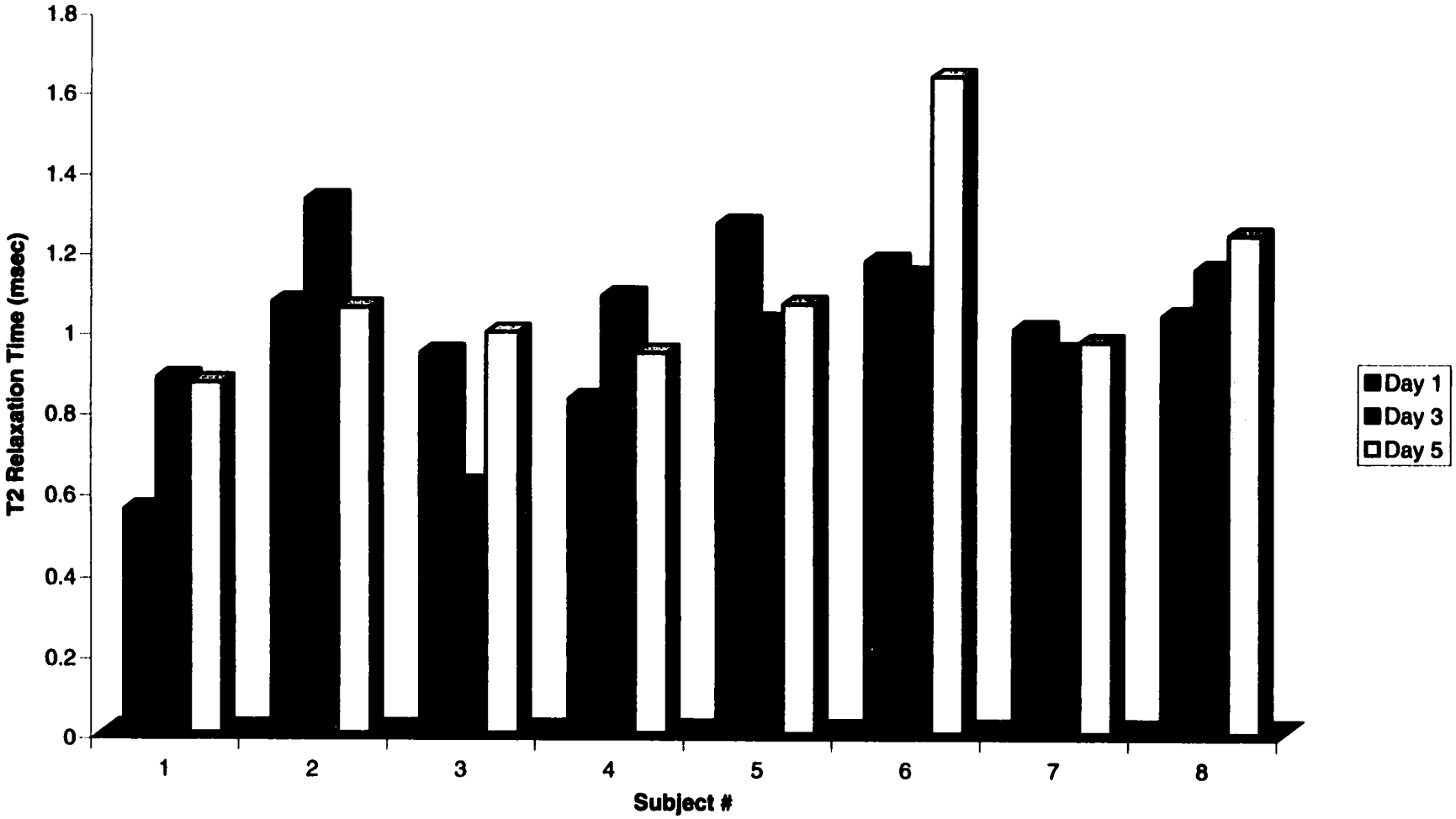


Figure U: Individual STIR Ratios over the 5 day testing period for the rectus femoris muscle (control group).

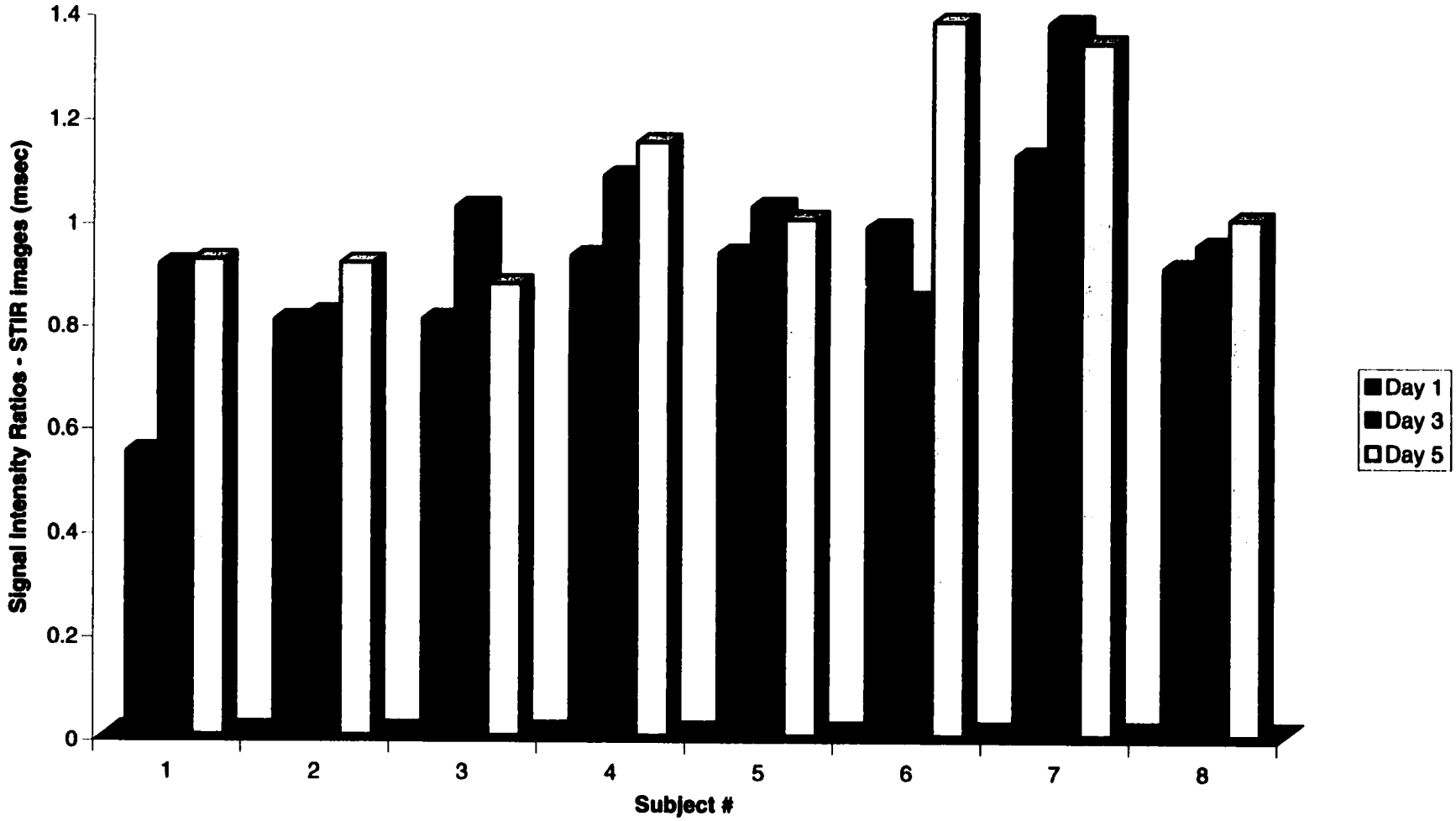


Figure V: Individual STIR Ratios over the 5 day testing period for the rectus femoris muscle (experimental group).

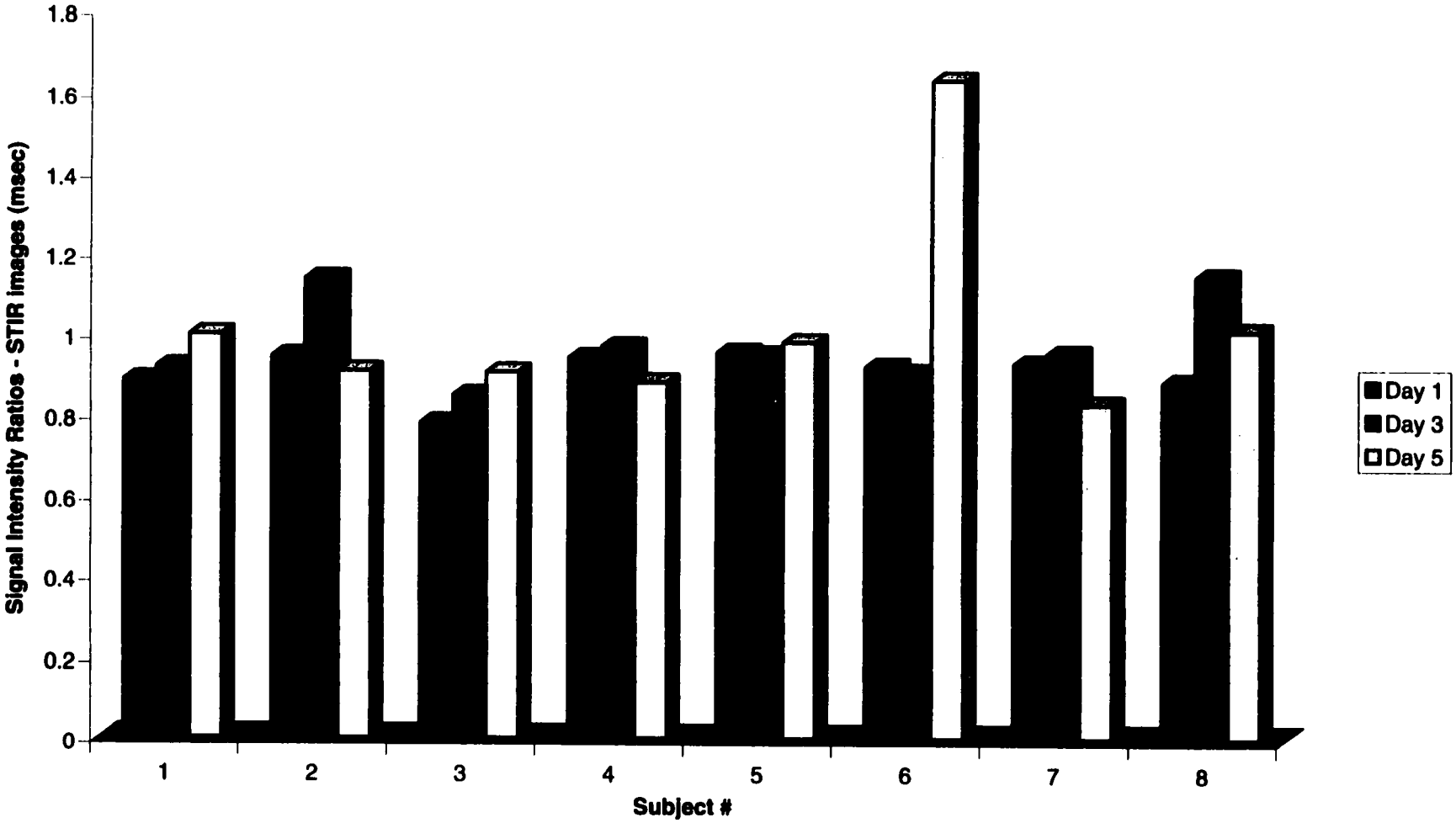


Figure W: Individual STIR Ratios over the 5 day testing period for the vastus intermedius muscle (control group).

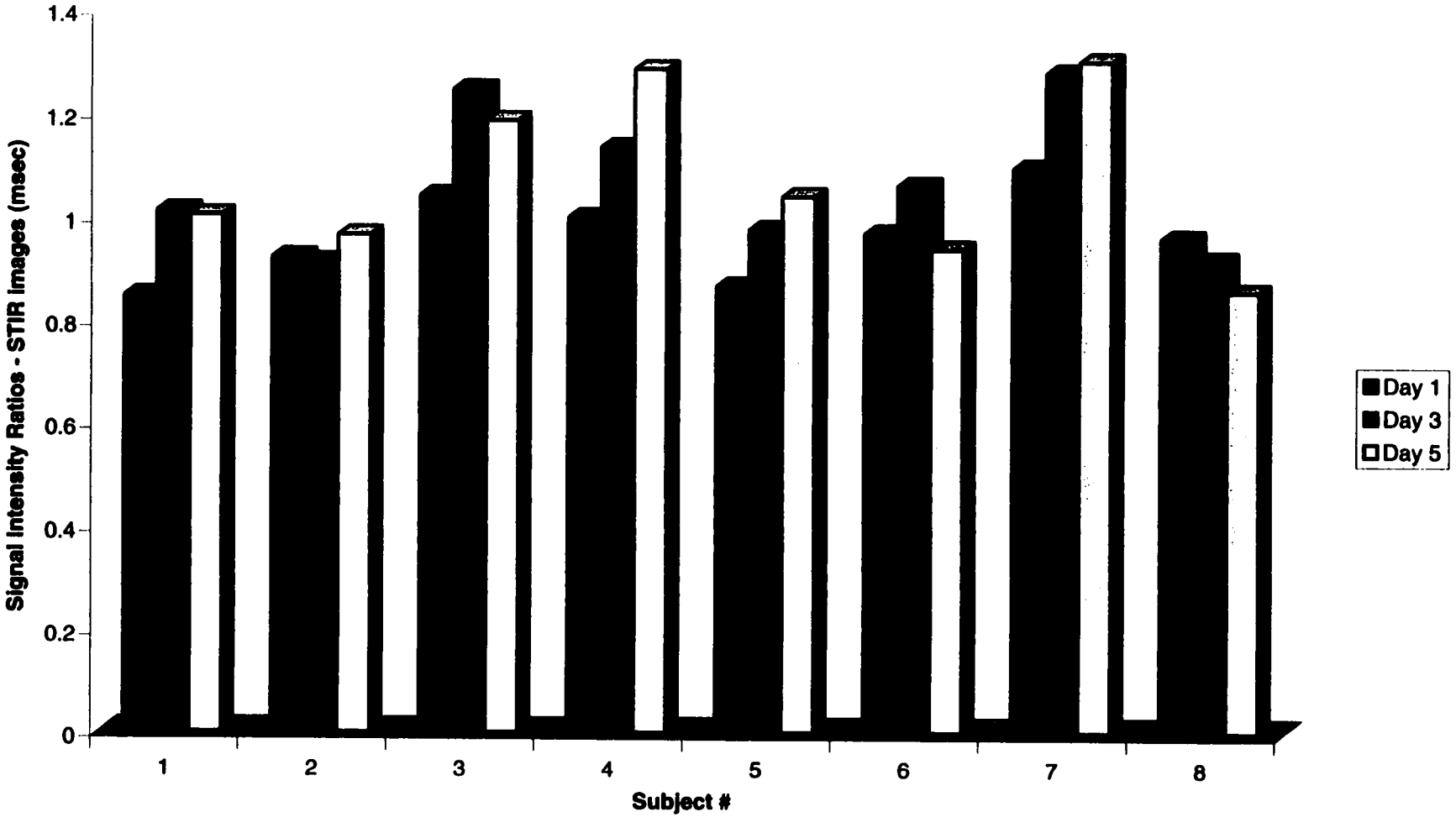


Figure X: Individual STIR Ratios over the 5 day testing period for the vastus intermedius muscle (experimental group).

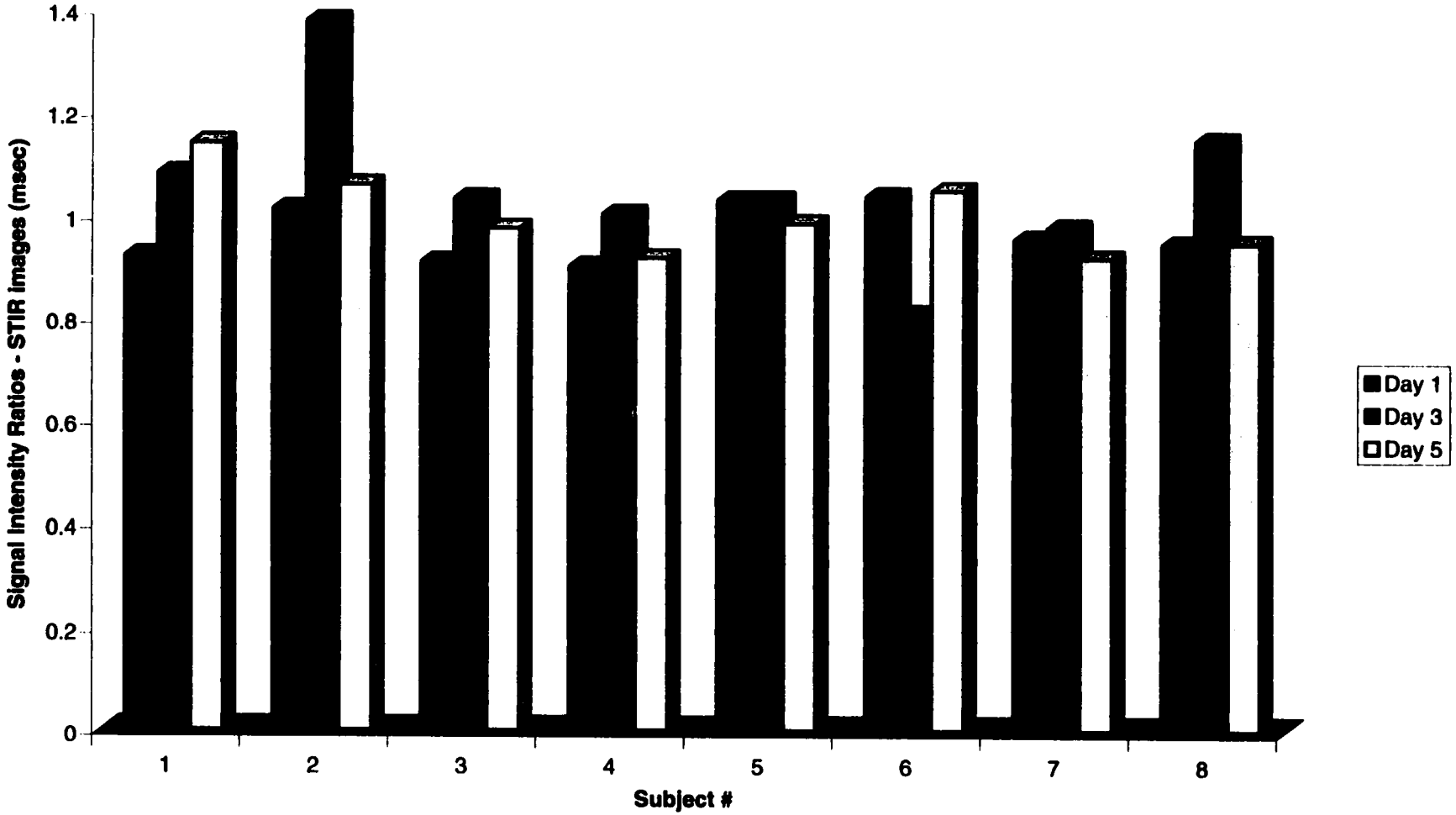


Figure Y: Individual STIR Ratios over the 5 day testing period for the vastus lateralis muscle (control group).

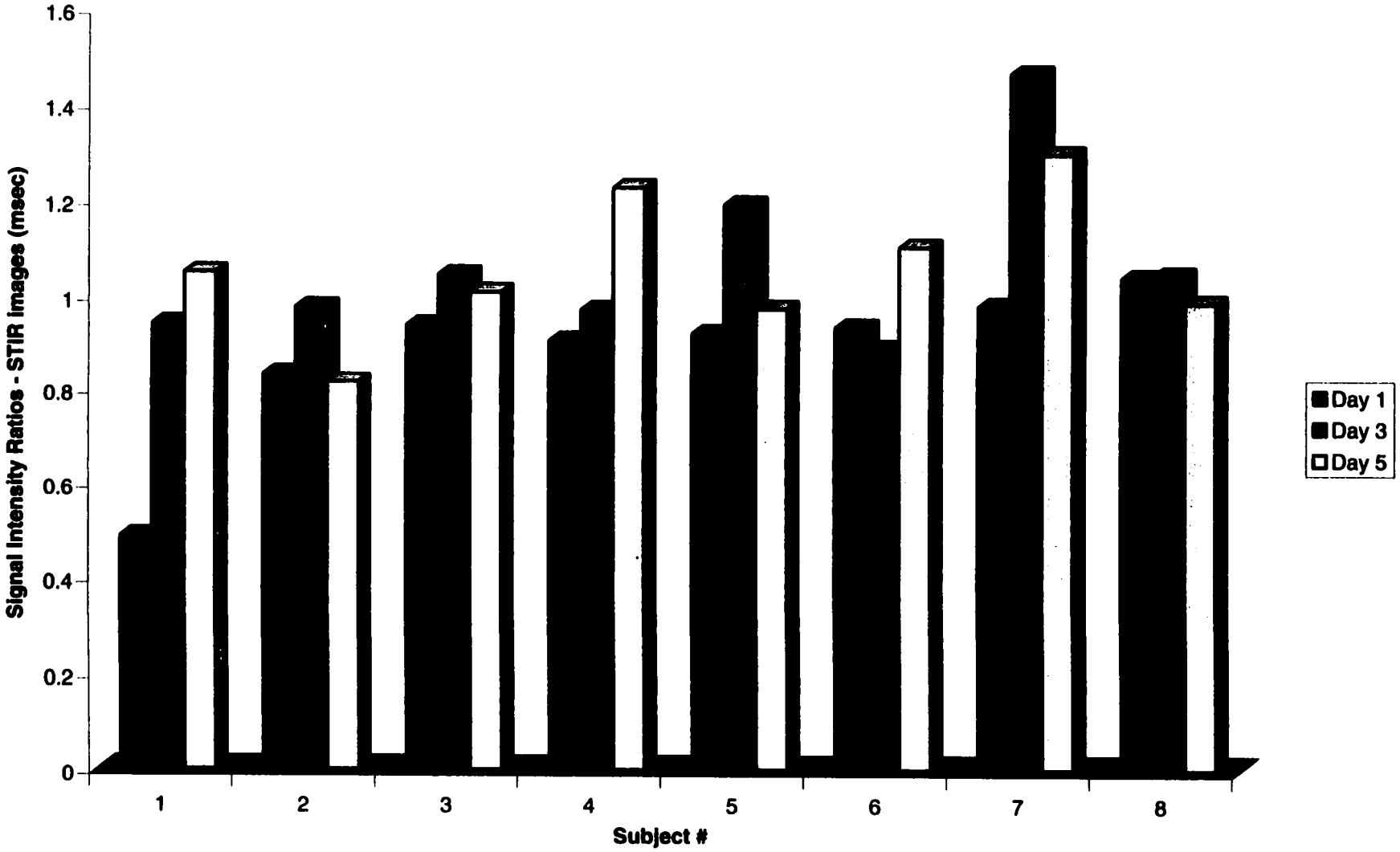
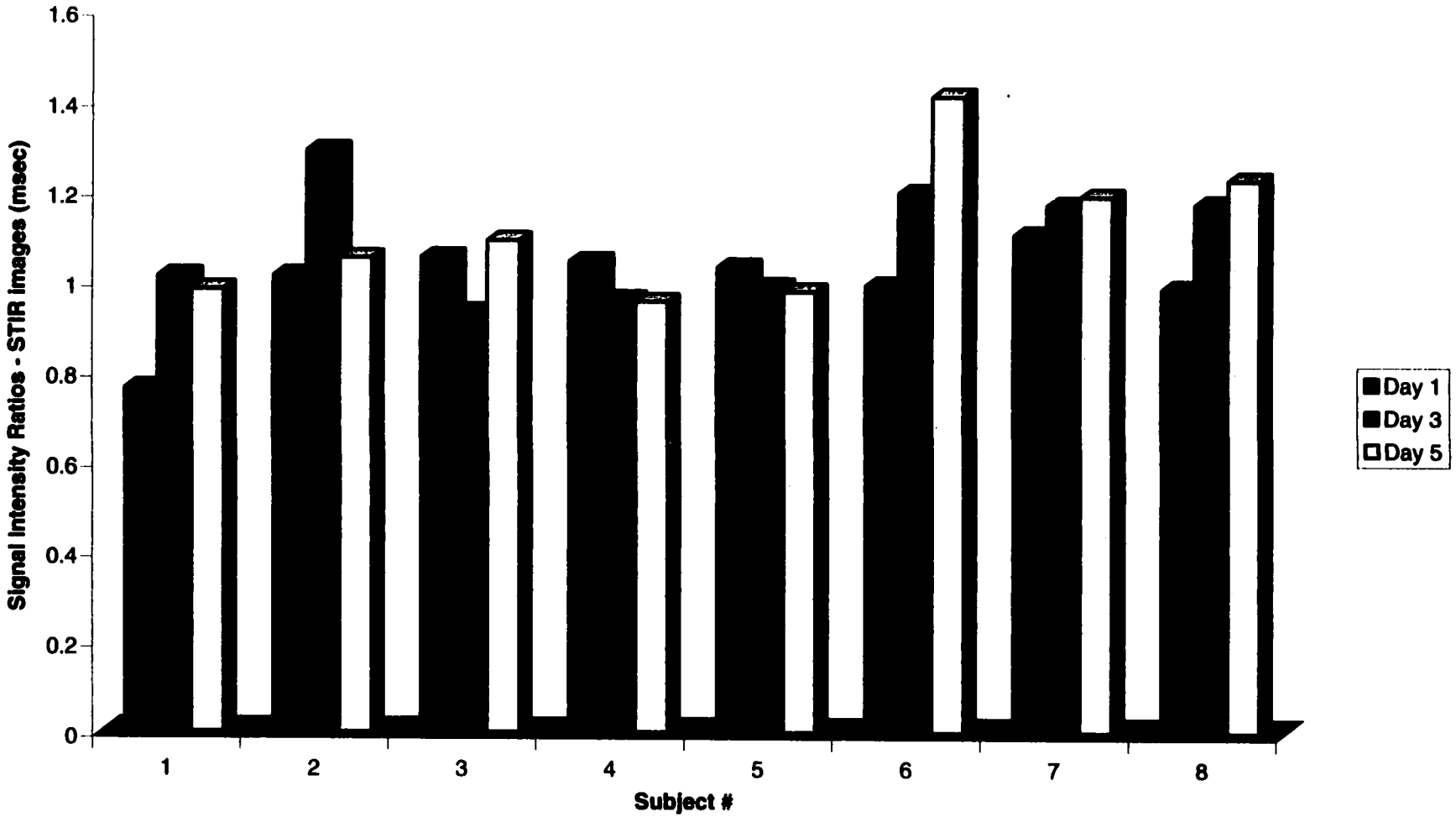


Figure Z: Individual STIR Ratios over the 5 day testing period for the vastus lateralis muscle (experimental group).

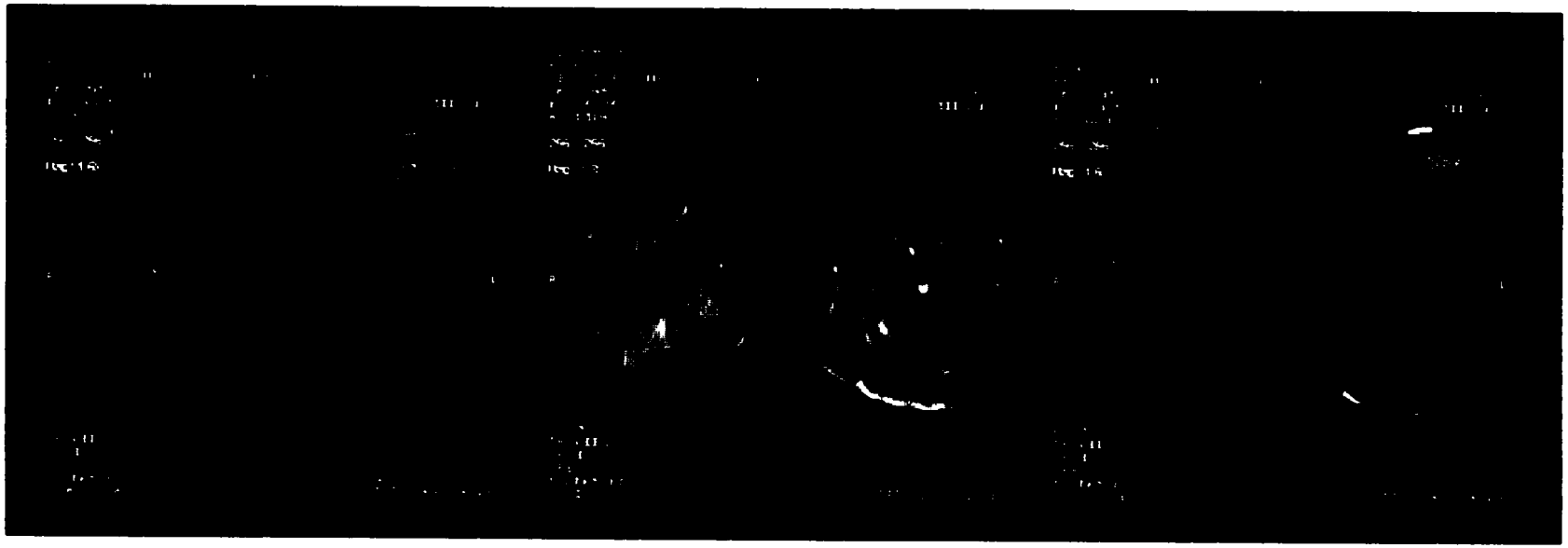


APPENDIX D: STIR IMAGES

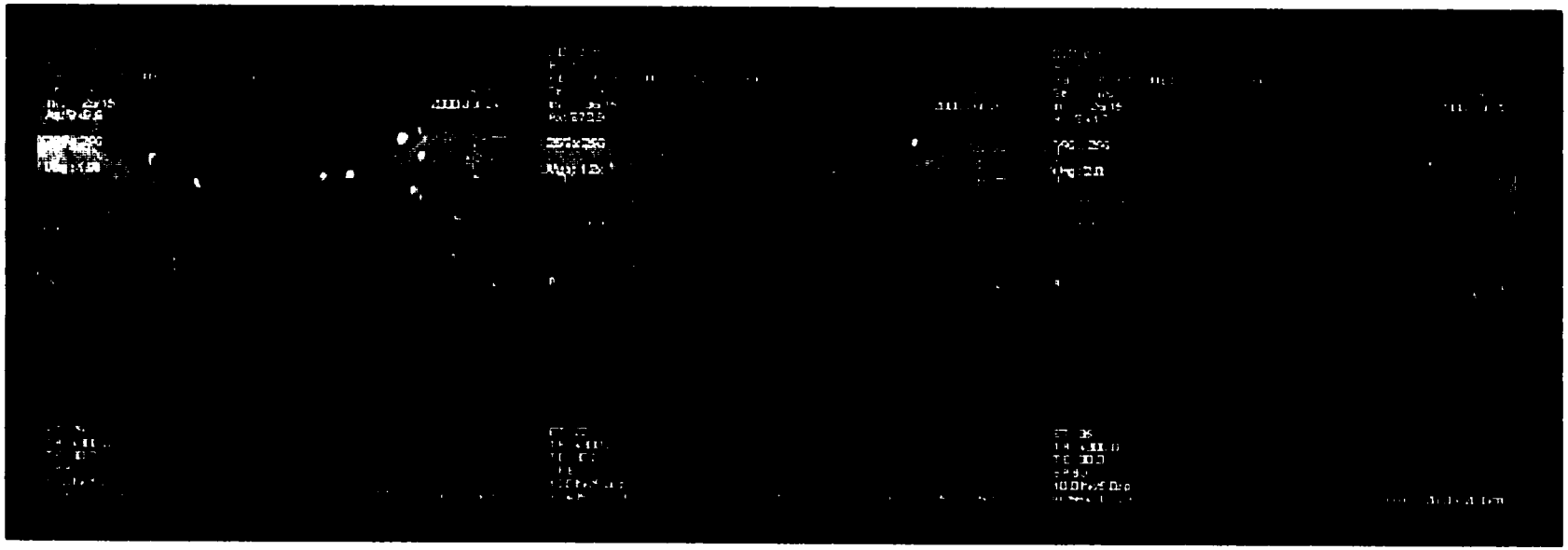
(DAY 1 [4 Hours Post-Ex], 3 [24 Hours Post-Ex], 5 [72 Hours Post-Ex])

ALL SUBJECTS

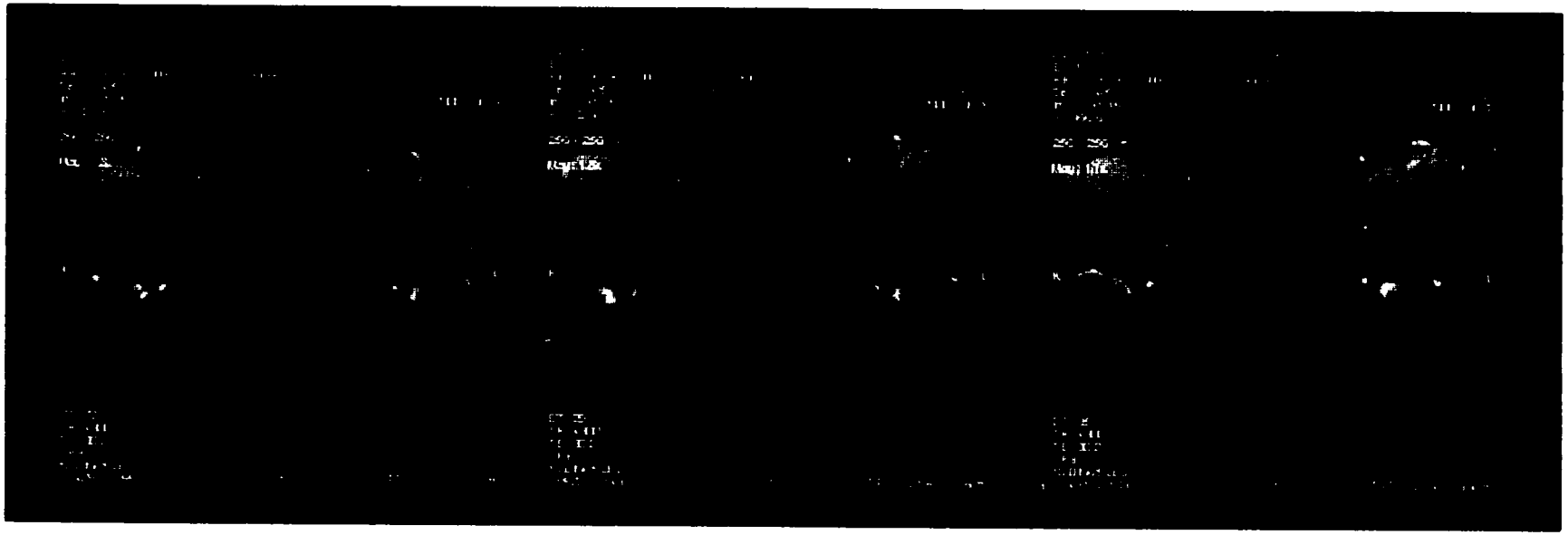
Subject #1: STIR images for days 1, 3 & 5



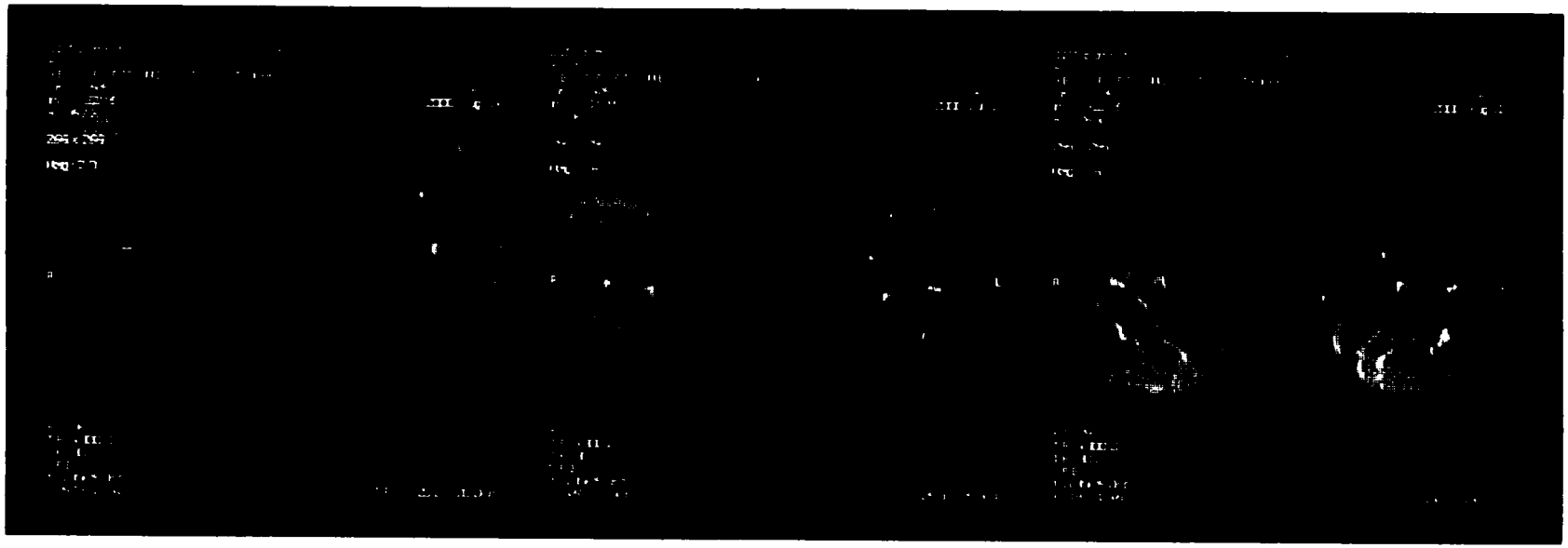
Subject #2: STIR images for days 1, 3 & 5



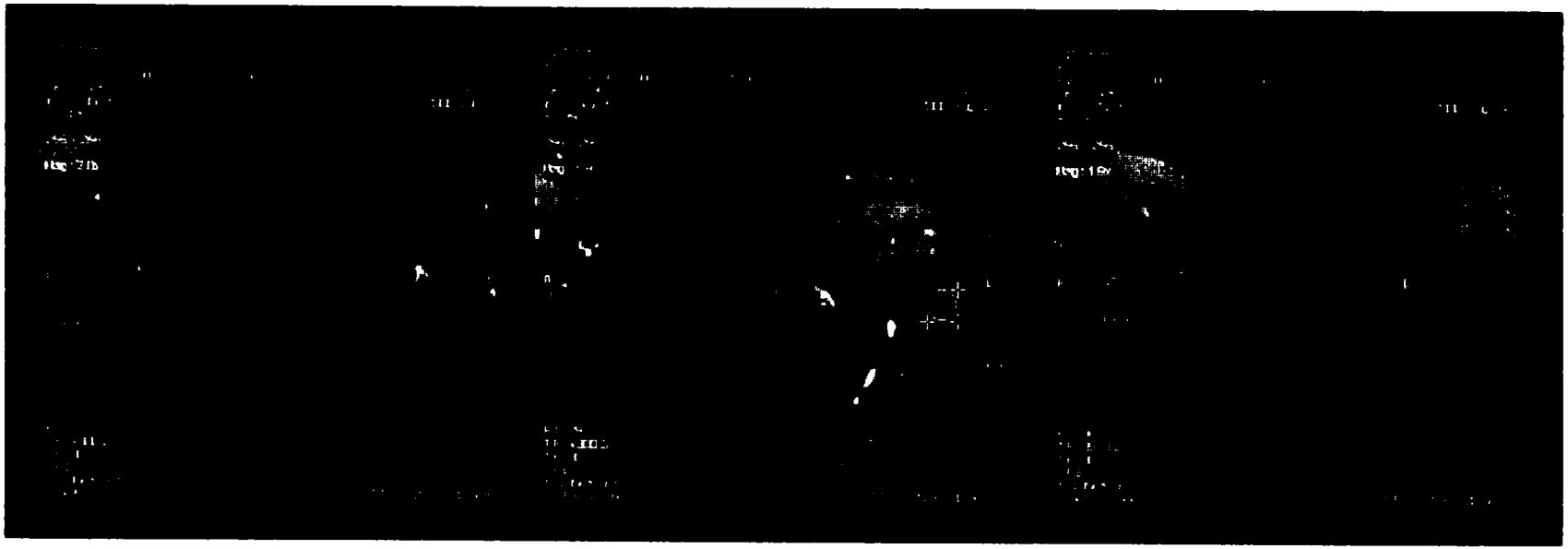
Subject #3: STIR images for days 1, 3 & 5



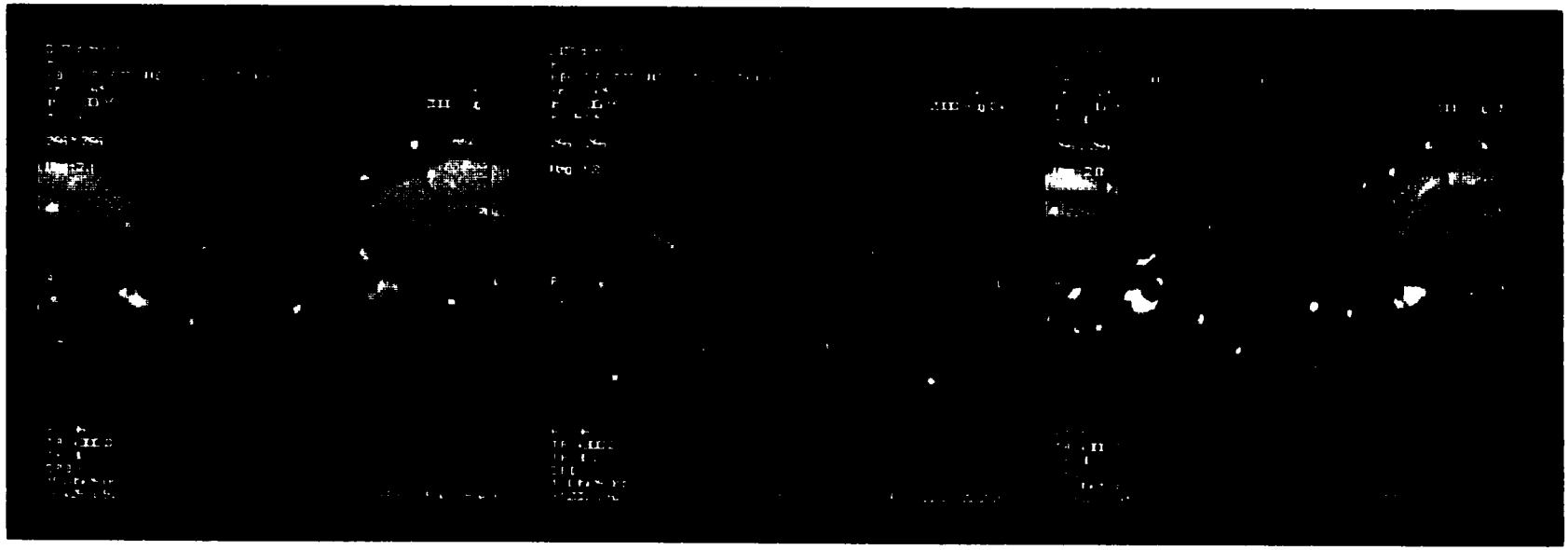
Subject #4: STIR images for days 1, 3 & 5



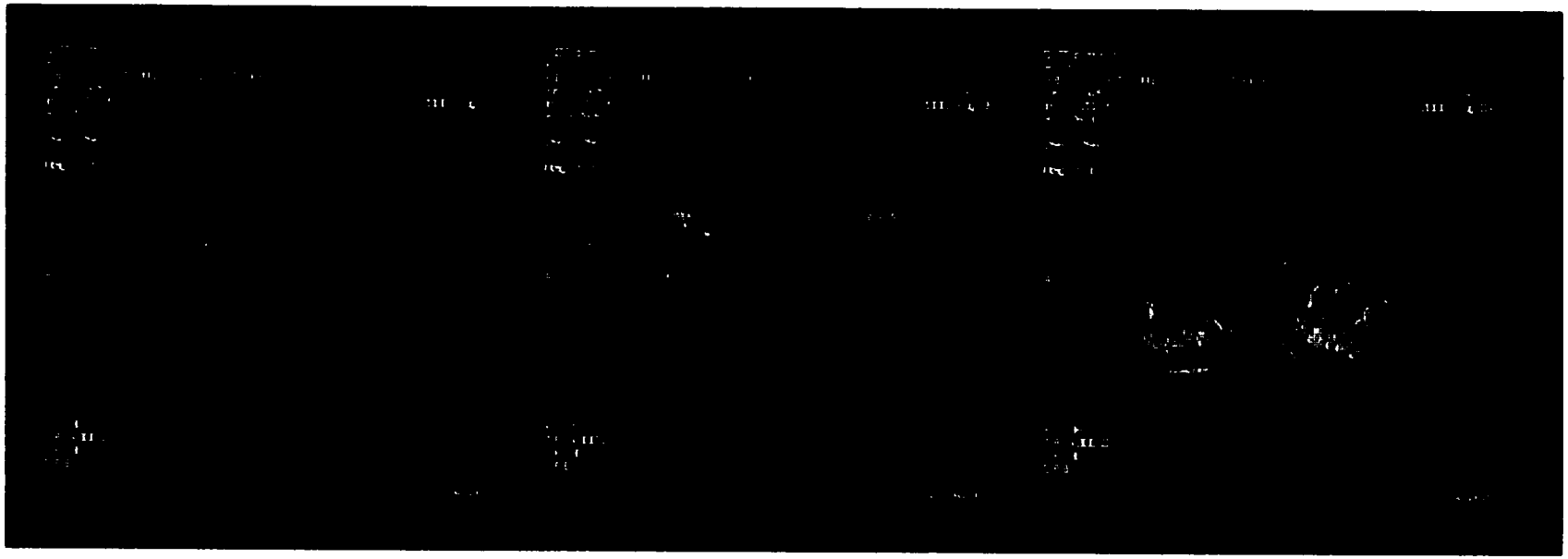
Subject #5: STIR images for days 1, 3 & 5



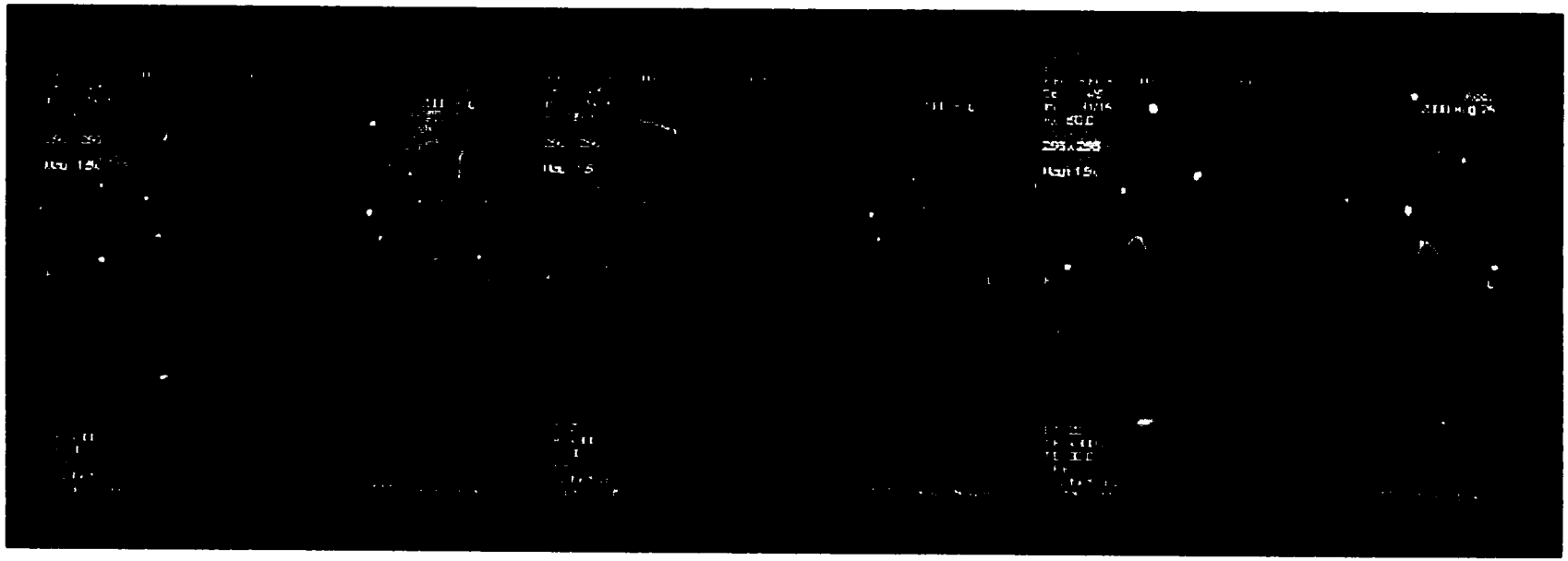
Subject #6: STIR images for days 1, 3 & 5



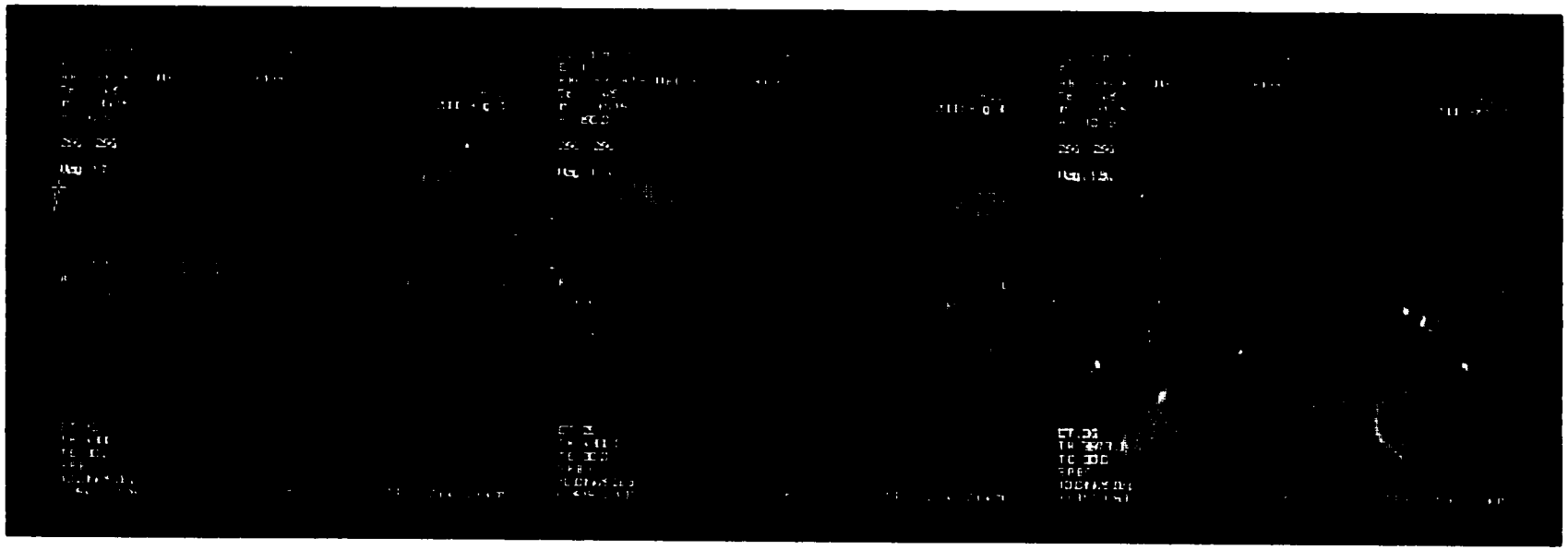
Subject #7: STIR images for days 1, 3 & 5



Subject #8: STIR images for days 1, 3 & 5

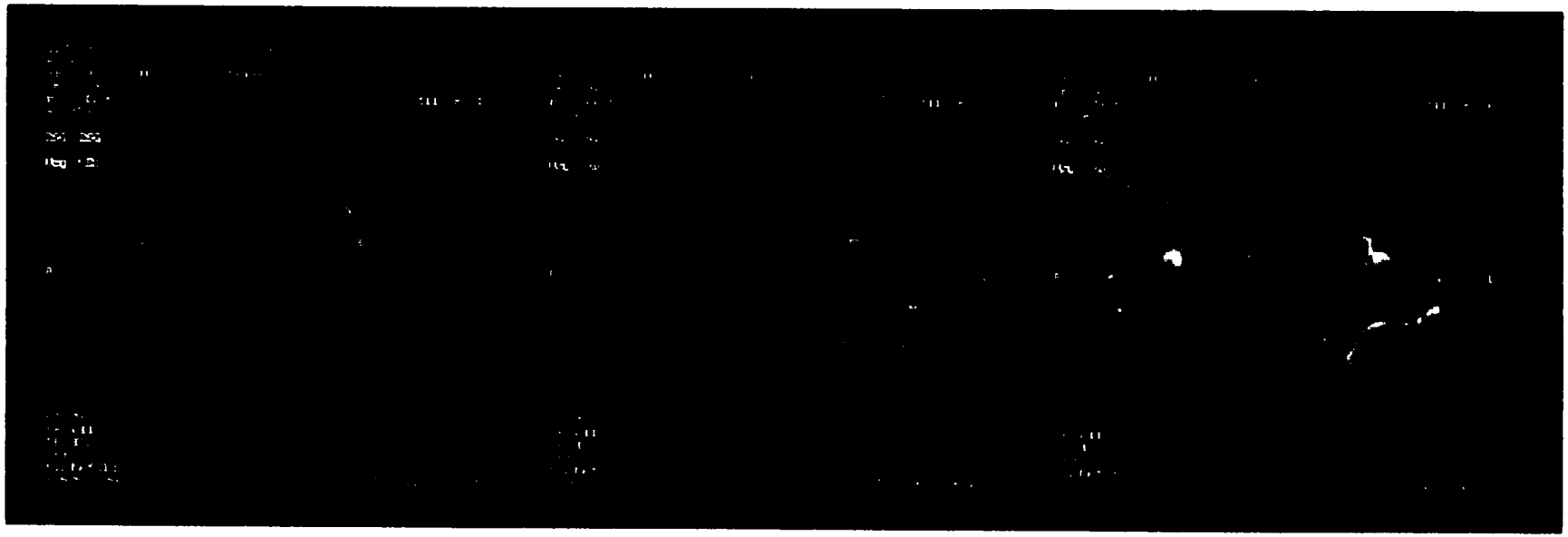


Subject #9: STIR images for days 1, 3 & 5

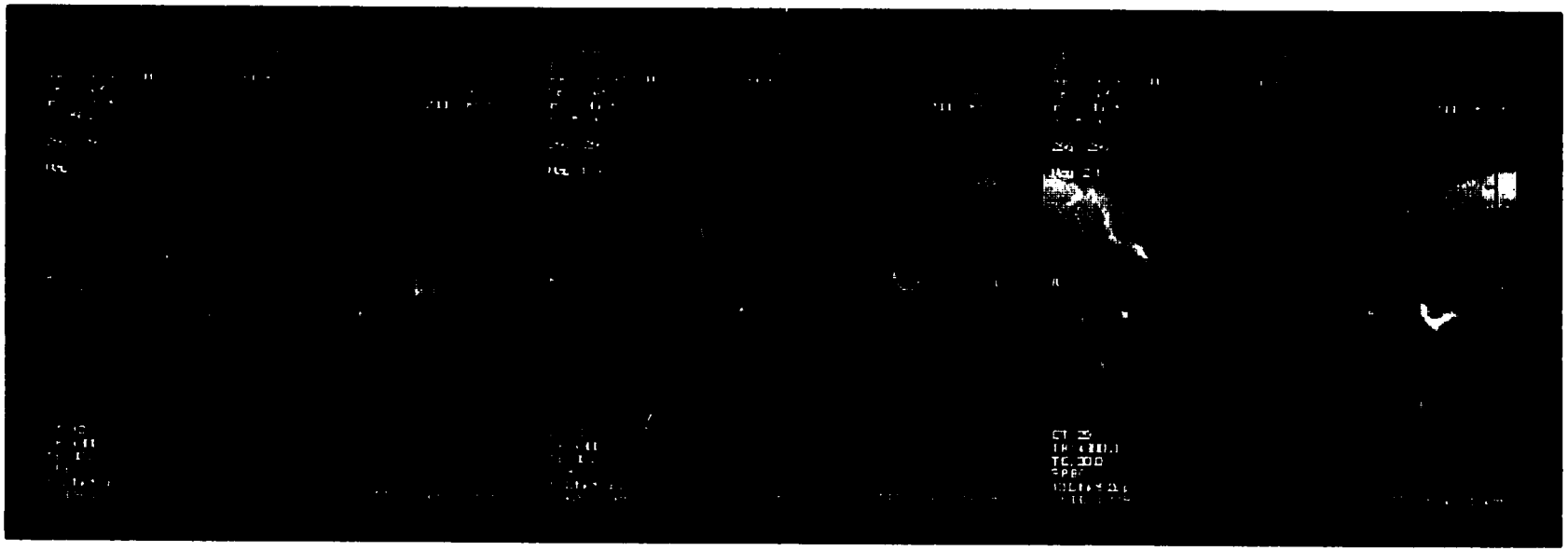


Subject #10: STIR images for days 1, 3 & 5

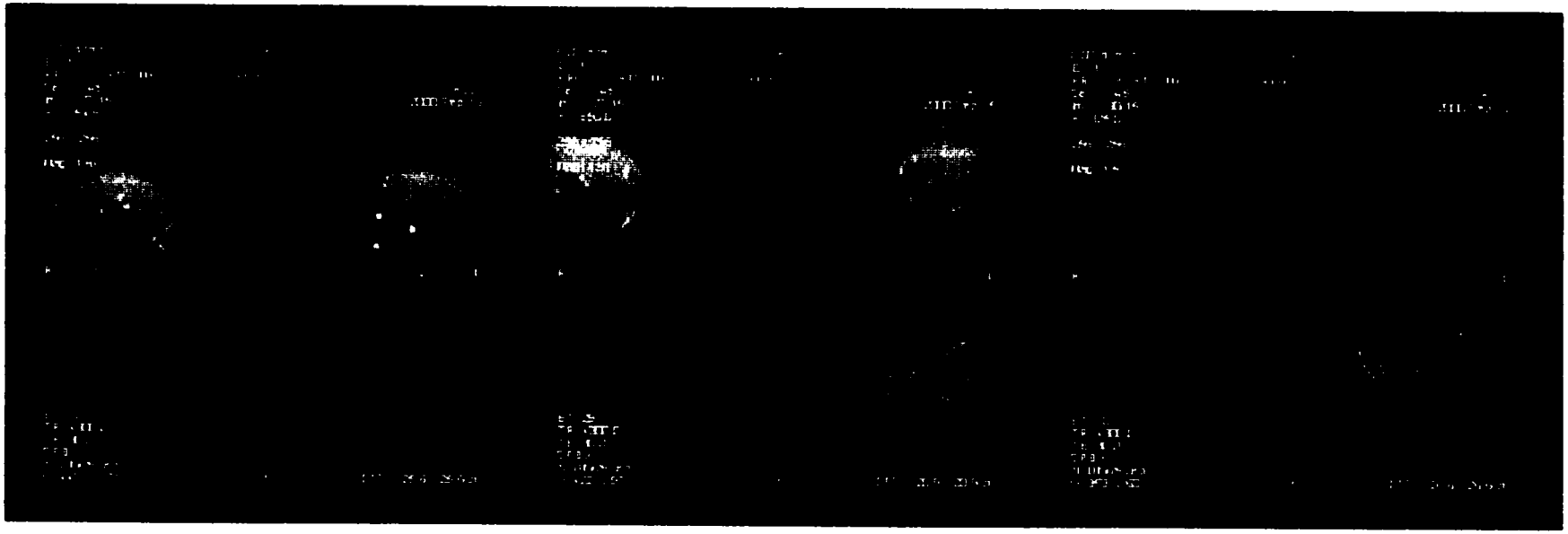
Subject #11: STIR images for days 1, 3 & 5



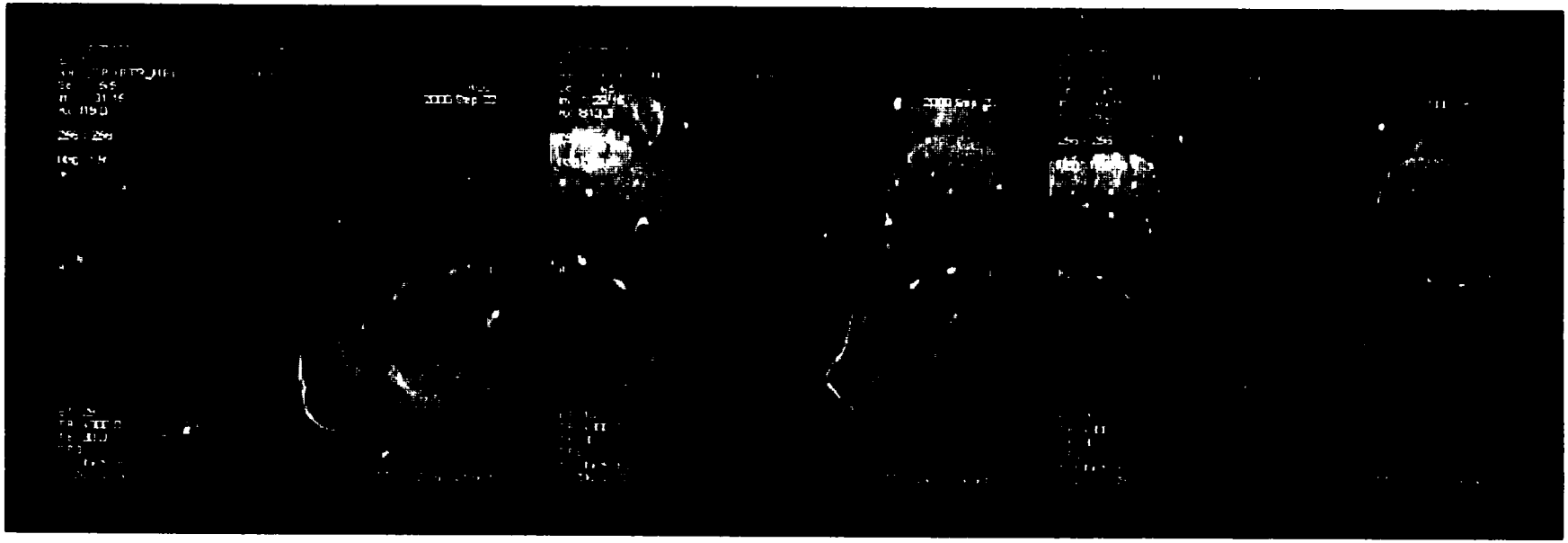
Subject #12: STIR images for days 1, 3 & 5



Subject #13: STIR images for days 1, 3 & 5



Subject #14: STIR images for days 1, 3 & 5



Subject #15: STIR images for days 1, 3 & 5

Subject #16: STIR images for days 1, 3 & 5