THE EFFECTS OF MASSAGE ON PERCEIVED PHYSICAL SORENESS, PAIN, AND MARKERS OF INFLAMMATION FOLLOWING HIGH INTENSITY UNACCUSTOMED EXERCISE

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TITLE: The Effects Of Massage On Perceived Physical Soreness, Pain, And Markers of Inflammation Following High Intensity Unaccustomed Exercise

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ABSTRACT

The Effects Of Massage On Perceived Physical Soreness, Pain, And Markers Of Inflammation Following High Intensity Unaccustomed Exercise

Courtney Lynn Crow

Massage is often recommended to athletes to facilitate recovery and attenuate DOMS. The purpose of this study was to investigate the effects of massage on perceived muscle soreness and pain, inflammatory and immune markers, ROM, and mood state. Fourteen, recreationally active, women participated in a randomized crossover design study, consisting of 1) 60 min. full body massage following unaccustomed exercise and 2) 60 min. of rest following unaccustomed exercise. Perceived muscle soreness and pain, active range of motion (ROM), mood state, along with blood concentrations of interleukin-6 (IL-6), C-reactive protein (CRP), creatine kinase (CK), and neutrophil count (NC), was assessed at baseline, 4hrs, and 24hrs following both treatment and control conditions. The aims of this study were 1) to decrease the effects of delayed onset muscle soreness (DOMS), and increase time to recover, and 2) to investigate the effect of massage vs. passive rest on inflammatory and immune markers within the blood. We hypothesized 1) an increase in ROM, a decrease in perceived physical soreness and perceived physical pain, as a result of the massage, compared to control, and 2) a decrease in blood plasma inflammatory markers, CRP, NC, CK, and IL-6, as a result of the massage, compared to control. We found massage following exercise to 1) significantly decreased perceived pain (p=0.001), 2) significantly increased immune
markers (WBC \( p=0.012 \) and NC \( p=0.012 \)), and 3) significantly decreased ROM \( p=0.02 \), compared to control. Massage had no impact on inflammatory markers (IL-6, CRP, and CK), or mood.

Key Words: Massage, Perceived Physical Soreness, Perceived Physical Pain, Delayed Onset Muscle Soreness, Interleukin-6, C-reactive Protein, Creatine Kinase, Neutrophil
ACKNOWLEDGMENTS

To My Husband:

Jeffery, you have been there every step of the way.

You push me, challenge me, and lift me up.

This journey would not have been the same without you.

Your endless love and support has helped guide me through this challenging adventure.

I could never fully express how much you mean to me. I love you!

To My Son:

Jack, you amaze me every day.

I push myself to succeed, to build a foundation, and to set an example for you.

No words could ever express my love for you.

To My Family:

Thank you for all your love and support. This would not have possible without it!
To Dr. Hagobian, Dr. Phelan, & Professor Olmstead:

Thank you for the knowledge and wisdom you have imparted.

The knowledge and skills I have gained in research would not be possible without your mastery and dedication to your students.

You set an amazing example for your students to live up to.

You have challenged me, pushed me, made me question and seek answers.

Thank you for your guidance, encouragement, and commitment.

To All My Professors:

I am truly grateful for my education, and this experience.

I take with me a foundation to build a life long edification, to which you have imported your own knowledge and wisdom.
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Chapter I
INTRODUCTION

Statement of the Problem

Athletes frequently experience delayed onset muscle soreness (DOMS) 7-72 hours after high intensity or unaccustomed exercise (16, 25, 50, 75, 76, 80), resulting in potentially negative effects on training regimens (69). Intensity and duration of exercise affects muscle damage and performance, decreasing range of motion (ROM), increasing swelling, and ultimately inducing DOMS (50, 69). Moreover, further evidence indicates that DOMS triggers a chain reaction of intracellular events, including an increased inflammatory response (e.g. interleukin-6 (IL-6), C-reactive protein (CRP)), mobilizing neutrophils (NC) into circulation, and elevation of intracellular enzymes (e.g. creatine kinase (CK)), which results in decreased muscle function (50, 75), and physical work capacity. Therefore, preventing and attenuating the inflammatory response may be beneficial during resistance exercise training.

Several modalities have been used, and recommended, to speed recovery and optimize training regimens, including low-level laser therapy (1, 49), cryotherapy (7, 78), analgesics (7), dietary supplementation (7, 79, 82), rehydration (2, 77), increased carbohydrate consumption (77, 79, 82), and dietary modifications (7, 82). Massage is an emerging field that has often been recommended for recovery, however empirical studies examining the beneficial effects of massage are needed (7, 31, 33, 84). Massage is often applied with the intent of increasing circulation and realigning stressed and overly
contracted muscle fibers to a more normal length (84). However, there is a lack of credible data to support this claim (73, 84). It is possible that the equivocal evidence results from inconsistent and poor study design, amongst other factors (See Table 1 below). For example, studies to date have utilized small sample sizes (61, 84), with questionable methods that make it difficult to generalize findings to a population; applying massage regionally as opposed to systemically (2, 23, 30, 31, 33, 52, 87, 88), lack independent control groups (2, 23, 33, 87), or lack a sufficient study design. See Table 1.1 below for summary of studies evaluating massage and exercise.

Despite the potential confounds, massage applied after exercise has been shown to decrease perceived muscle soreness (23, 35, 41, 76, 88), and speed recovery (23, 37, 39, 41, 52). For example, Farr et al. (2002), found that massage, relative to no massage, significantly reducing muscle tenderness (p<0.004), and significantly (p<0.004) increased muscle strength 72 hours post exercise (23). Similarly, Jakeman et al. (2010), found massage, relative to no massage significantly decreased time to recover with countermovement jump performance and squat jump performance 24 and 48 hours post exercise (p<0.01) (41). In addition, Zainuddin et al. (2005), found massage to decrease the severity of soreness by 20-40%, after a 10 minute massage three hours post exercise (88). Thus, preliminary evidence suggests that massage may improve performance, following damaging, unaccustomed exercise. However, several studies showed massage to have no effect on muscle function (35, 88). Consequently, there are too few studies, with inconsistent evidence to draw appropriate conclusions (23, 31, 35, 88).
In addition to improving performance, preliminary evidence suggests that massage may also decrease the systemic inflammatory response and speed recovery after exercise. For example, Hass et al. (2013), found that massage after exercise decreased immunohistochemistry in animals (p<0.0001) (32). Several studies have shown that massage after exercise decreased CK concentrations (marker of muscle damage) (37, 39, 76, 88) and decreased the inflammatory and immune response (IL-6, CRP, NC) (39, 76, 88), indicating less systemic inflammation. In contrast to these studies, others have found no beneficial effect of massage on inflammatory or immune markers (35, 41, 58).

Studies have not been comprehensive (see table 1.1 below). Differences in beneficial effects of massage may hinge on the time frame of massage given after exercise (76). Early intervention appears to be the most optimal strategy for minimizing tissue injury, facilitate tissue repair and recovery of muscle function (11). For example, Hass et al. (2013), found immediate massage-like compressive loading (MLL) was more beneficial in restoring muscle function compared to delayed MLL (32).

**Statement of Purpose and Research Hypotheses**

Therefore, the purpose of this study was to investigate the effects of massage on perceived muscle soreness and pain, and inflammatory and immune markers. We hypothesized that massage after exercise, relative to no massage, would 1) decrease perceived muscle soreness and pain 2) decrease inflammatory markers (CK, IL-6, CRP, NC), 3) increase range of motion (ROM), and 4) enhance mood.
Significance

Athletes are constantly seeking ways to increase performance and training volume. There is, however, the deterrent of physical soreness and limitation of muscle function after unaccustomed exercise, and thus a delayed return to peak performance, increased muscle soreness and pain, and delayed time to recover. The aim of this study was to investigate the effects of full-body massage on muscle soreness and pain, and to potentially shed light on speeding the recovery process and decreasing the effects of DOMS. While there has been some research on post exercise massage and muscle recovery, the results have been inconsistent. Post exercise massage may alleviate inflammation and symptoms related to DOMS, and thereby speed recovery. Thus, this study may increase knowledge and understanding of how massage may increase the recovery process after unaccustomed exercise.

Delimitations

We selected a convenient sample of female CalPoly students, between the ages of 18-30 to allow for on-campus blood draw, and to control for consistency in pressure and application of massage for the treatment group.

Definition of Terms

Delayed onset muscle soreness (DOMS): is a sensation of pain, stiffness, and discomfort that can be detected 7 to 72 hours after exercise as a result of unaccustomed exercise or large increases in volume of exercise (16, 22, 50, 75, 76, 80). Symptoms of
exercise induced muscle damage include increased soreness, swelling, weakness, structural damage, limb volume, and circumference, and decreases in muscular strength, power output, and ROM (22, 76). The symptoms of DOMS are mediated by the inflammatory response, causing an influx of cells and fluids in the affected muscle to remove damaged debris before cellular regeneration begins (22).

Inflammatory response: Inflammation describes a set of symptoms; heat, redness, pain, and swelling (11), and results from a cascade of events of white blood cell activation and recruitment, which is initiated through chemical, thermal and mechanical stressors (11), (e.g. injury to muscle, bone, and associated connective tissue) (11, 76). It is thought that secondary damage could result from neutrophilia within the 24 hours after exercise (11, 50). The release of cytokines like IL-6 increase the production of CRP (56, 20) and lead to neutrophil activation, and subsequent cytotoxic substances, followed by reparative macrophages (11). In addition, metabolic muscle disturbance is thought to cause a depletion in adenosine triphosphate (ATP) resulting in leakage of calcium into intracellular space, changing cell permeability, allowing leakage of cell contents into circulation (4). Muscle membrane disruption is considered to be the initial event leading to muscle injury, leading to neutrophil activation (11), therefore being an important mediator causing muscle damage (80) and subsequent inflammation. Muscle injury, resulting from strenuous unaccustomed exercise, and subsequent cytokine activation, contributes to the inflammatory response (11, 44). The role of inflammation, however, is not fully understood (11).
**Unaccustomed Exercise:** Unaccustomed exercise is a novel activity (35), with increased intensity or duration of exercise (22). Increased damage generally occurs at higher intensities and with unfamiliar actions (16).

**Interleukin-6 (IL-6):** A key pro-inflammatory cytokine in the inflammatory response (66), with ranges from ~1 pg/ml in resting healthy subjects to 10,000 pg/ml in individuals with severe infection (27), and has been found in skeletal muscle (66, 27), and systemic concentrations during the recovery phase of exercise (27). Exercise-induced muscle damage has been thought to be the primary stimulus for IL-6 response (8, 44). The chain of events resulting from muscle damage, increased macrophage entry into the muscle, resulting in further production of IL-6 (47). Mode, intensity and primarily the duration of exercise determine the magnitude of plasma IL-6 concentration (27).

**C-Reactive Protein (CRP):** Is a non-specific systemic inflammatory marker (20, 47, 56), produced by hepatocytes in response to elevations in IL-6 (56). CRP is responsible for the clearance of infected and the necrotic material (56). It is postulated that CRP binds to phospholipids of damaged cells to enhance uptake by macrophages (44). Additionally, it has been shown that regular physical activity reduces levels of CRP (56).

**Creatine Kinase (CK):** An intracellular enzyme and marker indicating muscle membrane damage (8, 69), muscle cell necrosis or disease (4). CK can range from 20 to
16,000 U/L in the general population, with levels above 5,000 U/L typically indicating serious muscle disturbances (4). When strenuous activity occurs, damaging the muscle cells, membrane permeability changes (18), releasing CK into the interstitial fluid, and is subsequently picked up by the lymphatic system and released into circulation (4, 9). Strenuous exercise that damages muscle tissue results in an increase in serum CK (4, 9, 18, 50, 69, 75, 80). CK typically peaks within 24 hours after exercise and then gradually returns to baseline over several days (9, 18, 75).

**Neutrophil:** Neutrophils have been shown to be the first white blood cells to enter damaged tissues and is required for the removal of damaged tissue (11). Thus, are involved in tissue injury and inflammation (70), including the inflammatory response following exercise (43). Muscle membrane disruption is considered to be the initial event that leads to muscle injury, followed by neutrophil activation and recruitment by muscle fibers and mast cells (11). Neutrophil activation occurs in response to an increase in epinephrine, and blood flow, that causes cell signaling molecules to move neutrophils away from the cell walls and into circulation (11). Exercise induced muscle damage results in membrane disruption and subsequent neutrophil infiltration (70, 80).

**Massage:** The application of mechanical pressures and manipulation of soft tissue, causing changes in parasympathetic activity (31, 84), decreasing tissue tension, and neurological excitability (84), increasing blood flow, facilitating fluid movement (33, 41,
83) and the removal of cellular debris, which influences the inflammatory process to speed recovery (41), and increases a sense of well-being (84).
Table 1.1: Summary of studies evaluating massage and exercise.

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Table 1.1: Summary of studies evaluating massage and exercise. Sample size (SS), Randomized counter-balanced crossover (RCB/CO), Randomized control design (RCD), Limb to limb comparison (LxL comp), Perceived physical soreness/pain (PPS/PPP) Creatine Kinase (CK), Neutrophil Count (NC), Interleukin-6 (IL-6), C-Reactive Protein (CRP), Muscle function (M Fx), Therapist training (Th Trn), Length of massage (Tm M), Years of experience (Yrs Exp), Full-body massage (FBM), Time-frame between exercise to massage (Tm BTW), Range of Motion (ROM), Physical Therapist (PT), Licensed Physical Therapist (LPT), Athletic Trainer (ATC), Trained Masseur (TM), Qualified Massage Therapist (QMT), Unknown (U), Professional Sports Masseur (PSM), Experienced Massage Therapist (EMT), Qualified Sports Massage Therapist (QSMT), Licensed Massage Therapist (LMT), Immediately (I)
Chapter II

LITERATURE REVIEW

Description of Massage, Inflammatory and Immune Markers and Exercise

The primary purpose of the study was to investigate whether massage had an effect on the blood bio-markers after high intensity exercise. To accomplish this, perceived physical soreness/pain, was assessed, along with blood concentrations of CRP, IL-6, CK, and NC, as a result of damaging unaccustomed exercise followed by massage. Below is a description of outcome variables assessed in this proposal.

Exercise and Delayed Onset Muscle Soreness

It is well documented that unaccustomed exercise, especially eccentric exercise, leads to DOMS (13, 16, 22, 43, 50, 67, 69). Paddon-Jones et al. (2000), stressed 20 non-resistant trained subjects with 36 maximal isokinetic eccentric contractions of the elbow flexors of the non-dominant arm. Perceived muscle soreness, assessed with a 12 point visual analog scale (VAS), significantly increased (p<0.001) following exercise, peaking between 24 and 72 hours post exercise (67). Lee et al. (50), assessed perceived soreness with a 10 point VAS, in eight healthy, untrained males. Subjects performed 60 eccentric contractions at the elbow, of their non-dominant arm, at 150% of their maximal isometric force production of the dominant arm. High intensity exercise significantly increased perceived muscle soreness 24-96 h following eccentric activity in the non-dominant arm.
(4.33 ± 0.73, 5.84 ± 0.94, 4.39 ± 0.77, 2.74 ± 0.37, at 24, 48, 72, and 96 hours, respectively) (50).

In addition, intensity and duration of exercise contribute to the degree of DOMS elicited, and may effect muscle damage and performance, decrease ROM, and increase swelling and inflammation (69, 15). Paschalis et al. (69), found high intensity exercise to induce a greater degree of muscle damage over low intensity exercise (p<0.05), showing that the same number of repetitions resulted in greater initial muscle damage and decrements in force output after high intensity exercise and faster recovery after low intensity exercise (p<0.05) (69). High intensity eccentric exercise is associated with an inflammatory response (10), commonly resulting in muscle soreness (50), and compromised muscle contractility and function (11), 24-48 h after exercise (10).

Unaccustomed exercise, typically eccentric exercise, is more likely to induce muscle damage over other forms of activity (4, 22, 64). Traumatized, swollen muscle fibers that have been damaged due to eccentric muscle activity are presumed to be weaker and more vulnerable to injury (18, 69, 75). It has been documented that concentric-only activity does not result in the same quantity of muscle damage as that of eccentric activity (64). Eccentric activity requires low muscle fiber recruitment, but greater volumes of force (11, 22), and high tension during movement (75), placing high mechanical stress on the system, disrupting the contractile component of the muscle, leading to increased muscle damage (11, 22). In addition, greater muscle damage has been seen in longer muscle fibers over shorter fibers (22). Nosaka et al. (2002), assessed the changes in muscle damage after 24 maximal eccentric contractions that followed an
eight-week training regimen of concentric exercise (in one arm) and eccentric exercise (in the other) (64). They found significant differences in maximal isometric force (MIF) between treatments and over time (p<0.01). In addition, MIF was significantly less in the eccentrically trained arm (30.5-49.3%; mean, 40.3 ± 5.6%) compared to the concentrically trained arm (15.6-21.2%; mean, 18.2 ± 2.4%), from immediately before exercise to immediately after. A four day follow-up, after the first training session, again showed MIF to be significantly less in the eccentrically trained arm (72.4% of before) compared to the concentrically trained (103% of before). Significant differences were not seen between treatments from immediately before to immediately after exercise on the four day follow-up after treatments two through eight (64). Likewise, Concentric and eccentric combined activity results in greater muscle damage and strength loss (~20-30%) than solely eccentric activity (~10-15%) (7). In addition, Byrne et al. (12), found no significant differences in muscular strength (p<0.05) between isometric, concentric and eccentric actions of the knee extensors, after a combined eccentric and concentric exercise of 100 barbell squats (10 sets of 10 reps) at 70% of the participants body mass. However, the squat jump performance decline was significantly greater than the countermovement jump performance (91.6 ± 1.1% vs. 95.2 ± 1.3%, p<0.05), following combined activity (12). Thus, type of muscle activity may result in the amount of muscle damage and consequently affect the level of performance.

The perceived physical soreness/pain associated with DOMS depends on the trained state of the muscle and can interfere with athletic preparation and performance (16) of novice and elite athletes, alike. Muscle recovery depends on age, gender, diet,
amount of sleep, training experience, and type of exercise (18). Likewise, inadequate recovery can result in further injury, as well as reductions in motor performance and unfavorable mental conditions (18), affecting peak athletic performance. In addition, increases in perceived soreness have been shown to reduce time to exhaustion, and decrease muscle strength, power and endurance (41). Proper recovery is associated with gains in performance (18), and is thus, essential to an athlete's success.

In review, the available literature suggests

- Unaccustomed exercise leads to DOMS, which persists for several days following exercise.
- Intensity and duration of exercise contribute to the severity of DOMS
- The associated symptoms of DOMS, including perceived physical soreness and pain, limit the adherence and return to physical activity and peak athletic performance.

**Exercise and Inflammatory Response**

**Inflammatory Response**

Exercise evokes an inflammatory and immune response, that persists for several days following unaccustomed exercise (43). For example, previous research has shown that unaccustomed exercise increases CK concentrations (4, 8, 9, 23, 41, 65, 70, 75, 76, 80, 88), increases IL-6 (27, 65, 70, 80), increases CRP (5, 30, 44), and increases NC (11, 70, 76, 80); all of which interfere with recovery from exercise.
The inflammatory response seems to be related to the type and amount of activity. Kasapis et al (2005). Reviewed several studies that showed marked increases in CRP, CK, and white blood cells immediately and 24 hours following intense physical activity (44). Weight et al. (85), Alexander, and Jacobs observed marked, but transient increases in CK (+800%, p < 0.01), CRP (+2000%, p < 0.01), and WBC (+160%, p < 0.01), immediately and 24 hours after a 42 Km marathon race. Another study by Taylor et al. (1987), found increases in CK (+1200%, p < 0.05), and WBC (+158%, p < 0.05), and CRP (+266%, p < 0.05) after 21 km of canoeing, 97 km of cycling, and 42 km of running (81).

IL-6 and CRP are both indicators of immune function (20). Immunoregulatory effects of exercise induce IL-6, and showed increases in CRP, inducing an anti-inflammatory response (27), thus showing CRP to be stimulated by IL-6 (44). Deviations within the muscle system during exercise activate pro inflammatory cytokine like IL-6, leading to endothelial activation and neutrophil attraction (11). In addition, it has been shown that NC can be mediated by release of IL-6, and seems to correlate to CK activity (70). Yet, neutrophils have been shown to release into circulation before CK, thus potentially being an important mediator causing muscle damage (80). In addition, previous research has shown that neutrophil mobilization and release of CK are attenuated by repeated daily exercise at the same “relative” workload (80). However, short-term repeated exercise activity, 3 consecutive days of cycling at 90W for 90 min., was not shown to attenuate IL-6 (p<0.01), or CK (p<0.01) for post day one to post day three (80). The increments of NC seen post exercise on day one (p<0.01), were not seen
post exercise on day three (80). Another study by Donges et al. (2010), assessed the effects of 10 weeks of resistance training, or aerobic training, compared to a control groups on IL-6 and CRP (20). No significant difference in IL-6 (P>0.05) was observed between the control or exercise groups, however significant reductions in CRP (32.7% ± 27.2%, p<0.05) was observed in the resistance training group (20). Thus history of repeated exercise may be key to attenuation of inflammatory markers, CRP and CK. History of exercise does not seem to affect attenuation of IL-6 and NC.

**Exercise and CRP**

Few studies have examined the effects of exercise training on CRP (20, 44, 47, 56). In addition, CRP expression has been shown to vary with types of exercise; joggers (odds ratio (OR) = 0.33) and olympic dancers (OR = 0.31) showed significantly lower values of markers of inflammation compared to weightlifters (OR = 0.83), swimmers (OR = 0.62), and cyclist (OR = 1.30) (44). After nine months of marathon training, Mattusch et al. (2000), observed a reduction in CRP levels by 31%, with no change observed in the control (54). While CRP increases dramatically with unaccustomed exercise, it is postulated that the decrease in CRP levels with regular exercise is the result of the reduction in the production of cytokines in muscle, fat and mononuclear cells, and an increase in insulin sensitivity, which improves endothelial function, and reduces body weight (44).
Exercise and IL-6

The response of blood concentrations of IL-6 after exercise is well documented (20, 27, 44, 50, 66). A study conducted by Ostrowski et al. (2000), showed marked increases in plasma IL-6 concentrations in trained athletes after a 2.5 hour treadmill run (p<10^{-14}), leading researchers to hypothesize the relationship of IL-6 to either running intensity or duration (66). A literature review of marathon runners, showed peak increases in IL-6 immediately after exercise, typically up to 100-fold, followed by a rapid decline (27), confirming an association between exercise intensity and plasma IL-6 concentrations (66). In the same study creatine kinase showed a slight increase (4-fold) immediately after exercise and peaked 24 hours after the marathon (16-fold), showing no association with IL-6. Thus suggesting that muscle damage was not the stimulus of IL-6 (66). Plasma IL-6 concentrations, however, increase in response to exercise (27, 50, 66).

IL-6 has been found to have an inflammatory controlling role for the return to homeostasis (66). While there is little research, IL-6 concentrations have shown to be lower in trained subjects after exercise, compared to non trained individuals (27). Thus there seems to be a link between sedentariness and systemic inflammation (20).

Exercise and Creatine Kinase

Several studies have looked at the effects of exercise on CK (4, 8, 9, 18, 70, 75). Smith et al. (1994), found that an additional bout of exercise during the time frame of DOMS does not alter the time frame of serum CK (P=0.074), DOMS (P=0.962) or decrements in strength (P=0.106) (75). Brancaccio et al. (2007), note that exercise
duration has more to do with serum CK levels over fitness level (9). The highest post exercise serum CK levels were found after prolonged exercise that includes eccentric activity, (e.g. ultra-distance marathon running, triathlon events, weight-bearing exercises, and down-hill running) (8, 9). Thus, there a direct relationship between the increase in CK levels and the level of cellular necrosis (9). In addition, CK is the catalyst for reversible phosphorylation of creatine to phosphocreatine and ADP to ATP, and is therefore found in muscle tissue as well as in circulation (4), further suggesting the involvement in cellular necrosis.

Total CK levels depends on age, gender, race, muscle mass, level of physical activity and climatic conditions (4, 9). Men having higher levels of CK before and after exercise, blacks over caucasians, resting CK is higher in athletes over sedentary subjects, cold weather over warm (9). CK response after exercise is typically lower in trained athletes compared to untrained (8, 9), with most marked changes occurring in less trained subjects. This could be the result of less muscular damage due to routine training, resulting in greater muscle mass (9).

**Exercise and Neutrophil Dynamics**

Neutrophil activation is thought to be the catalyst in muscle injury after exercise. Recent studies have shown when neutrophil activation is blocked, a significant decrease in early tissue damage results (11). The reparative processes of macrophages is similarly inhibited when neutrophils are blocked, slowing phagocytosis of cellular debris, as well as regeneration and repair. Thus, some neutrophil function is necessary for tissue repair
(11). Limiting, as opposed to blocking neutrophil activation may reduce tissue damage and enhance tissue repair (11). Consequently, it would seem that proper regeneration of tissues is dependent upon communication between neutrophils and macrophages (11). As a result, secondary damage could be due to neutrophilia within the 24 hours following exercise (11, 50).

In review, the available literature suggests

- Inflammatory response is related to amount and intensity and duration of exercise.
- Total CK levels depends on age, gender, race, muscle mass, level of physical activity and climatic conditions.
- Neutrophils are an important mediator in the inflammatory response.
- IL-6 is not affected by repeated exercise overtime, suggesting that it could be an initiator of inflammation.

*Massage and Mood*

Massage has been shown to improve mood, mind clarity and enhance a sense of wellbeing. Field et al. (1996), assessed the effects of 15 min. of chair massage twice weekly for five weeks on POMS (depression and anxiety) questionnaire, math computation, and saliva cortisol levels (n=26), compared to a control group (n=24) (26). Both massage and control group showed significantly lower POMS depression score following the first and last sessions (p<0.01). However, only the massage group showed a significant decrease in state anxiety from the first to the last session (p<0.001). The
massage group showed a significant decrease in the time to completion (p<0.01) for the math computation along with a significant decrease in the number of errors (p<0.001). In addition, the massage group showed statistically lower levels of cortisol on day one, where the control showed significant increases following the last session (p<0.01) (26). Weinberg et al. (1988) assessed the relationship of 30 minutes of moderate intensity exercise (60% of max HR) (jogging, swimming, tennis, and racquetball), massage (30 min. full-body Swedish), and passive rest, on positive mood enhancement (86). POMS questionnaire was completed prior to, immediately following and 30 min following activity. Both running and massage groups showed significant decreases in tension (M=11.5 ± 7.9 to M=8.0 ± 5.8) and (M=6.8 ± 3.8 to M=1.6 ± 1.1), confusion (M=8.5 ± 5.3 to M=5.1 ± 4.5) and (M=4.7 ± 3.2 to M=2.7 ± 2.1), fatigue (M=8.4 ± 6.3 to M=6.6 ± 5.4) and (M=6.4 ± 5.3 to M=2.7 ± 2.3), depression (M=11.5 ± 8.1 to M=4.5 ± 8.4) and (M=5.2 ± 2.0 to M=1.5 ± 1.4), and anger (M=8.6 ± 8.4 to M=4.6 ± 6.3) and (M=3.0 ± 1.9 to M=0.7 ± 0.9), pre to post, respectively (86).

While it has also been shown that massage improves mood along with a sense of well-being (35), few studies have examined the relationship of massage, mood, exercise, and DOMS. Hilbert et al. (2003), assessed the effects of 20 minutes of localized Swedish massage following eccentric and concentric activity of the right hamstring (35). Subjects completed a POMS questionnaire 6 and 24 hours following exercise activity. They found massage to have a main effect (p<0.05), but no interaction. Post hoc analysis showed mood disturbances for both groups 24 hours following exercise (35). It has been argued, however, that the effects of massage are more psychologic than physiologic (35).
Conflicting results necessitates further research to determine the effects of massage on mood following the effects of DOMS.

**Massage and Exercise**

Several studies have examined the effects of massage on recovery after exercise (6, 7, 23, 33, 34, 35, 41, 61, 62, 76, 83, 84, 88). In general, most studies have shown massage to alleviate perceived physical soreness (23, 35, 41, 76, 88), yet few have shown a decrease in the time to recover, or an attenuation of the inflammatory response (76). Hilbert et al. (2003), assessed whether Swedish massage affected ROM, NC and perceived physical soreness, with a total of 18 subjects in two groups, after 6 sets of 10 maximal eccentric contractions of the right hamstring, followed by 5 additional maximal eccentric contractions of the right hamstring (35). Subjects received 20 minutes of localized massage (classic Swedish), arriving 2 hours after exercise, including effleurage and petrissage, compared to control. Post hoc analysis showed ROM decreased significantly for both groups at 24 and 48 hours post exercise from baseline. No significant differences were observed for ROM, NC or perceived physical soreness for treatment across time. A significant decrease in perceived physical soreness, however, was observed in the massage group at 48 hours post exercise (p<0.05) (35). Farr et al. (2002), studied the effects of therapeutic massage on DOMS and muscle function, after a 40 min. down-hill continuous treadmill walk, followed by a 30 minute massage on one randomly selected leg, 2 hours after exercise (n=8) (23). They found massage to be effective at reducing muscle tenderness (p<0.004), and showed a quicker recovery to
baseline in muscle strength for the massage group at 72 hours, compared to the control at 120 hours (p<0.004) (23).

Several studies showed massage to have no effect on muscle function. For example, Zainuddin et al. (2005), found no significant differences in ROM between treatments, after a 10 min. massage, 3 hours post exercise; utilizing an isokinetic dynamometer for 60 maximal eccentric contractions of the elbow flexors (n=10) (88). In addition, Hilbert et al. (2003), assessed whether 20 min. of localized Swedish massage affected ROM, after eccentric activity of the right hamstring (35). No significant differences with ROM were observed for treatment across time (p>0.05) (35).

Massage may also decrease the systemic inflammatory response of blood biomarkers and speed recovery after exercise. For example, Hass et al. (2013), found that massage after exercise decreased immunohistochemistry in animals (p<0.0001) (32). In addition, Hiruma et al. (2005), found a significant increase in CK levels (p<0.05) for the treatment group over the control, with a total of 14 subjects in 2 groups, from day two to day five of recovery, following bilateral calf raises, which ceased when participant could no longer maintain the indicated constant speed (37). Imtiyaz et al. (2014) accessed CK levels following 30 eccentric movements of the elbow flexors at 80% of their maximum isometric force, and massage or vibration therapy, compared to a control, with a total of 45 subjects in 3 groups (39). Significant differences in CK (p=0.002) were seen 48 hours following exercise for both the massage and vibration groups, compared to the control (39). In addition, Zainuddin et al. (2005), assessed the effects of a 10 min. massage (randomly assigned to one arm) on CK levels three hours post exercise, which consisted
of 60 maximal eccentric contractions of the elbow flexors on a isokinetic dynamometer (88). Peak CK levels were 36% lower in the massage group compared to the control (88). Smith et al. (1994), examined the effects of a 30 minute localized sports massage, within 2 hours following eccentric exercise, on CK, and NC, compared to control group (76). The massage group showing consistently lower ratings of CK (1917 ± 1023 vs. 1083 ± 476), and NC (4250 ± 351 vs. 3866 ± 554) showing a prolonged elevation with the massage group compared to control (76). Hilbert et al. (2003), assessed NC following damaging eccentric activity and localized massage, compared to control (35). No significant differences were seen for NC for treatment across time (p<0.05) (35).

The time frame of massage given after exercise may be critical to its effectiveness (76). For example, Hass et. al (2013). found immediate massage-like compressive loading (MLL) to be more beneficial in restoring muscle function, with 129% difference in peak torque recovery, compared to delayed MLL, with 82% difference (32). Smith et al. (1994), found 30 min. of localized sports massage, given within 2 hours following exercise, to have consistently lower ratings of perceived muscle soreness, in the massage group compared to the control group (n=14) (76). In addition, Zainuddin et al. (2005), found massage to decrease the severity of soreness by 20-40%, and peak CK levels by 36% after a 10 min. massage, applied 3 hours post exercise (88). Hart et al. (2005), however, found no treatment interaction for limb girth or pain, and no main effects, after three treatment sessions, consisting of girth and discomfort analysis, then five minutes on the stationary bike, followed immediately by a five minute sport massage, applied 24, 48 & 72 hours, following eccentric activity (n=19) (33).
These data suggest early application of massage may reduce neutrophil migration and levels of CK, subsequently reducing inflammation and associated DOMS (76). Yet, varying treatment times and the vast and complex sequence of techniques (23, 33, 35, 41, 76, 88) make replication challenging. In order to be effective, it has been suggested that massage should be applied one to three hours following activity (76). In addition, the massage may need to be longer, and applied within a specific time frame following exercise, in order to have an effect.

Level of training of the massage therapist may play a role in the response to massage, as many studies do not indicate the level of training or hours of massage application (23, 31, 33, 35, 41, 76, 88). For example, only indicating massage is applied by a “trained masseur” (23), “qualified massage therapist” (88), “qualified masseur” (31), “professional sports masseur” (41) “certified athletic trainer” (33), or “licensed physical therapist” (76), or “physical therapy student” (35). Certifications equate to an understanding of anatomy, not hands-on experience working with clients. Consequently it is possible that studies utilized insufficiently trained massage therapists (61), or practitioners that spend very little time utilizing massage techniques on clients (e.g. athletic trainers). In addition, training requirements for Massage Therapists change state to state. For example, California has two levels of certification and requires as little as 250 hours of education (14), where New York requires a minimum of 1000 hours (65). The limited research accessing the effects of training on massage outcomes suggests experience may be key to the results of application. For example, Moraska (2007), assessed the level of training of Massage Therapists, utilizing 450, 700 and 900 hours of
training (62). Deep effleurage was the primary technique utilized, however therapists were not bound to a protocol. Twelve to fifteen min. massage was applied within 60 min. of event completion. Questionnaire was used to assess perceived recovery. Results showed student therapists with 950 hrs of training had greater reductions in muscle soreness compared to student therapists with 450 hrs or 700 hrs (p<0.01) (62). Thus, the level of knowledge, understanding and hands-on experience of the practitioner may be key to the outcome of the massage.

It is clear that massage significantly decreases perceived physical soreness, however, the mechanism is unclear. It could be physiological or psychological, with most studies to date indicating a psychological component. Massage may be psychologically beneficial for individuals, increasing a sense of wellbeing, potentially from an increase in the release of endorphins (34). On the other hand, the pain and discomfort of DOMS potentially physically deters individuals from peak output or even a return to activity. By increasing the blood flow with massage, an increased clearance of cellular debris may slow or inhibit the inflammatory process and speed the recovery of function.

In review, the available literature suggests

• Education level of Massage Therapist and time period of application of massage might be critical to its effectiveness.

• Time frame of application, length of massage, type of strokes and depth of pressure may alter the effectiveness of massage.
• The limb to limb comparison may be a confounding factor; affecting participants perception of the actual effectiveness of applied massage.

**Conclusion**

Massage is commonly encouraged to speed recovery after intense physical activity. While several studies have shown massage to decrease perceived physical soreness, too many studies show equivocal and inconsistent results with inflammatory markers and performance outcomes. Unaccustomed exercise elicits DOMS, along with CRP, CK, IL-6 and NC. If massage can limit the activation of neutrophils, and IL-6, it may decrease the CRP and CK response and subsequently decrease the effects of DOMS.
Chapter III

METHODS

Overview

The goal of this study was to assess the effects of massage on perceived muscle 
soreness and pain, and inflammatory and immune markers, after unaccustomed exercise 
in women. A crossover study design was used where subjects served as their own 
control, completing both conditions, allowing results to hinge on application of massage. 
Additionally, the participants were counterbalanced, having participants randomly 
assigned to start with one of two conditions: 1) exercise and control (n=6) or 2) exercise 
and massage recovery (n=8). Participants completed the different conditions on two 
different days, with a minimum two week washout period between the two condition.
Perceived physical soreness (PPS), perceived physical pain (PPP), range of motion 
(ROM), emotional wellbeing, and blood biomarkers; interleukin-6 (IL-6), C-reactive 
protein (C-RP), creatine kinase (CK), and neutrophil count (NC), were assessed at 
baseline and 4, and 48 hours post unaccustomed exercise.

Subjects

Fifty-one females expressed interest in the study. Fifteen subjects met eligibility 
requirements following elimination of inclusion and exclusion criterion. One subject was 
lost to attrition, and their data was eliminated from analysis. Thus, fourteen healthy, 
recreationally active female students (22.11 (2.31) kg/m2; 20.93 (1.69) yr), were
recruited from CalPoly, San Luis Obispo University Campus. Inclusion criteria included: (1) 18 to 30 years, (2) BMI (18.5 to <30), (3) good health, assessed by a health history questionnaire, (4) non-smokers, (5) not pregnant, or trying to become pregnant (6) able to participate in regular physical activity (3-5 days/week), assessed by PAR-Q, (7) free of metabolic disease. Exclusion criteria included (1) any physical ailments or wounds that would prevent them from participating in physical activity or massage, (2) subject that received regular massage (more than once every three months), (3) subjects that took any medications or supplements, and (4) subjects who regularly participated in forms of high intensity physical activity. Since males tend to be larger with more muscle mass than females, this study only assessed women in the normal weight to overweight category for BMI so the Massage Therapist could ensure an adequate and consistent amount of force applied during the massage. Participants were informed of all risks, benefits and requirements of the study, and gave verbal and written consent. All documentation and procedures were approved by the Human Subjects Committee at California Polytechnic University, San Luis Obispo, California.

Screening and Informed Consent

A general overview of the study was provided to women who express interest in the study. To determine eligibility, they completed a brief telephone interview, including the Physical Activity Readiness Questionnaire (PAR-Q), to screen participants for their ability to engage in exercise. Eligible respondents came to the Human Performance Laboratory in the Kinesiology Department to discuss the study. During this visit, the PI
or research assistant conducted a brief screening to determine whether women were pregnant or trying to become pregnant. All pregnant women or women trying to become pregnant were excluded from the study. Once eligibility was confirmed, the study was explained in detail, including the importance of attending all assessment visits. The study and consent form was then reviewed and informed consent was obtained. Women who agreed to participate in the study were asked to complete the preliminary tests and study treatments.

Preliminary Tests

Subjects completed a Physical Activity Readiness Questionnaire (PAR-Q), Health History form, and a brief familiarization of the equipment and procedures. Height, weight, body mass index (BMI), and blood pressure (BP), were assessed for each subject, as perviously described, followed by preliminary baseline bench press and back squat session to estimate their one repetition maximum (1-RM) described below (46). All subjects were asked to refrain from exercise and alcohol 24 hours prior to any and all exercise sessions.

Experimenters

All exercise testing was conducted by a CPR certified Kinesiology Masters student of CalPoly San Luis Obispo, and overseen by a seasoned researcher. All massage was performed by a California Certified Massage Therapist, with 11 years of experience.
All blood samples were drawn by the CalPoly Health Center Lab, and all samples were analyzed by Central Coast Pathology.

**Bench-Press and Back-Squat Estimation and 1-RM Protocol**

To estimate bench press and squat one repetition maximum (1RM), we used the Brzycki equation (1RM = 100* load kg / (102.78 - 2.78* repetitions) (63) and established protocol of Kemmler et al. (2006), (46). After a 5 minute warmup on a stationary bicycle the subjects selected a weight that they thought they can lift, using good form (to 90 degrees), to fatigue within 6-10 repetitions, on a Hammer Strength Smith Machine Squat Rack, and Hammer Strength Adjustable Bench (Pro Style), LifeTime Fitness, Rosemont IL. If the subject failed to perform within the specified range the test was repeated within 72-hours (46). The participant then tested their 1-RM determined by Brzycki equation. Subjects performed eight to ten reps at 50% of their estimated 1RM on squat rack and bench press (73), followed by a three minute (3 min) rest period (59, 60). The weight was then be increased to 80% of their estimated 1RM, where the subjects completed one lift (73), again followed by a 3 min rest period (59, 60). The weight was then be increased to a failed attempt. The 1RM was reached within five attempts. The heaviest weight lifted to completion with good form was recorded for 1-RM (73).

**Experimental Protocol**

Subjects were asked to consume 16-20 oz of water the night before the 1RM protocol. In the morning after an overnight fast, subjects completed a baseline blood
draw, range of motion (ROM) assessment, emotional wellbeing using the Profile of Moods State (POMS), and completed perceived physical soreness and perceived physical pain questionnaires, using the Descriptor Differential Scale (DDS) and Numerical Pain Rating Scale (NPRS) (see below of detailed description) (68), followed by 1RM testing.

On week later, subjects again were asked to consume 16-20 oz of water the night before the experimental trials. In the morning after an overnight fast, subjects again completed ROM, POMS, DDS, and NPRS. Subjects were then randomized, in a counterbalanced order, to begin with one of two conditions; exercise and passive rest (n=6), or exercise and massage treatment (n=8). Subjects then were asked to consume 8-10 oz of water 15 minute prior to activity, and a baseline weight to determine hydration status was taken. Subjects then completed a 5 minute warmup on a stationary bicycle, and then subjects performed eight to ten reps at 50% of their 1-RM on a squat rack and bench press (73), followed by 3 minute rest period (59, 60). The weight was then be increased to 90% of their 1RM, and subjects completed a series of back squats and chest presses (6 sets of 8 reps at 90% of 1-RM) (35). Each set was separated by a 3 minute rest period (59, 60). Another body weight measurement was taken, following the exercise protocol. The massage condition received a 60 min full body massage, on a MasterWorks Massage Table, MHP International, Skokie, IL, within 20 min. of the completion of the exercise protocol. The subjects began the massage prone with a generalized foot massage (5min), followed by massage of the legs prone, including gluteal muscles (5 min per leg), massage of the back (15 min), and massage of the arms (2.5 min per arm). The client then turned supine and received massage of the anterior legs (5 min per leg), massage of
the arms (2.5 min per arm), and massage of the chest, neck, shoulders and scalp (10 min).

Massage strokes consisted of effleurage (stroking), and petrissage (kneading) strokes that engaged the muscle (staying between a four and a seven on the NPRS, used for pressure (0-no pressure to 10-unbearable pressure). The subjects were asked to indicate if they were uncomfortable at any time, for any reason, during the massage. The control condition consisted of 60 min of rest, on a MasterWorks Massage Table, MHP International, Skokie, IL, within 20 min. of the completion of the exercise protocol, utilizing similar environmental conditions as the massage group. There was a two week washout period between conditions to minimize any learning effect of the exercise protocol. Within four hours and 24 hours after unaccustomed exercise, all outcomes measures were collected again (blood draw, ROM, and perceived soreness and pain). All participants were asked to refrain from any and all anti-inflammatory interventions, including ice and NSAID use throughout periods of assessment.

**Physical Soreness and Pain**

Perceived physical soreness (PPS) and perceived physical pain (PPP) were both measured at baseline, 4 hours and 24 hours following onset of unaccustomed exercise, using a Descriptor Differential Scale (DDS), and a 0-10 Numeric Pain Rating Scale (0 no-pain to 10 unbearable pain), as previously described (68). An overall score for the DDS, for each assessment point was determined by averaging the rated value of intensity (45). See Appendix for questionnaires.
**Blood Draw**

Blood was drawn by a trained phlebotomist at CalPoly Health Center, and processed by Central Coast Pathology Laboratories at baseline, 4 hours and 24 hours following onset of unaccustomed exercise, to assess Interleukin-6 (IL-6), Creatine Kinase (CK), C-Reactive Protein (C-RP), and Neutrophil Count (NC).

**ROM Testing**

Pain free active range of motion (ROM) for the hip and shoulder (flexion, extension, adduction, abduction, internal and external rotation), knee and elbow (flexion and extension), was measured at baseline, 4 hours and 24 hours following onset of unaccustomed exercise, using a goniometer, taking the total average of 3 trials for each testing session (39).

**Emotional Wellbeing**

Mood and emotional wellbeing, was assessed at baseline, 4 hours and 24 hours following onset of unaccustomed exercise, utilizing a Profile of Moods State (POMS) questionnaire, consisting of 65 questions, with a rating scale of 0=not at all to 4=extremely for best descriptor of feelings (35). Total Mood Disturbance (TMD) is determined by adding values for Tension, Depression, Anger, Fatigue, Confusion, and subtracting Vigor (55). See Appendix for questionnaires.
Hydration Status

To ensure proper hydration of participants throughout testing we followed the ACSM recommendation guidelines. Subjects were asked to consume 16-20 oz of water before bed, the night before activity. They were then be asked to consume 8-10 oz of water 15 minute prior to activity, on the morning of testing. Weight was then taken using a Detecto 439-Physicians Scale before exercise, immediately following exercise, and 24 hours following exercise. Participants were weighed with minimal clothing with any sweat soaked clothing removed, to accurately access sweat loss (74).

Research Location

All exercise took place in the CalPoly Recreation Center Gym. All participants received massage and participated in active rest in a treatment room of the Kinesiology building at CalPoly.

Participant Debriefing

Subjects received study findings by email before publication, or in person, after study completion. They also had the option to attend a presentation on the findings and ask any questions they had before the final completion of the write-up and publication.

Analysis

SPSS software was used for statistical analysis of the data. All data was reported as mean ± standard deviation. A Repeated Measures ANOVA was used to assess
condition x time interactions for perceived soreness and pain, inflammatory and immune markers (IL-6, CRP, CK, NC), ROM, and mood state, adjusting for height, weight, BMI, and 1RM for squat and bench press. An alpha \(< 0.05\) was considered significant, and post-hoc test were used to determine the location of any differences, and to examine the differences in changes from baseline, and between time points.

**Sample Size Calculation**

The sample size for this study was based on Zainuddin et al. (2005), which showed a decrease in perceived muscle soreness for the massage condition compared to the control after exercise (42.9 ± 5.6 vs. 52.8 ± 7.0) (88). With a randomized crossover design, using a two sided t-test, \(\alpha<0.05\), with a sample size of 14 we will have 98.5% power to detect a statistically significant reduction in perceived muscle soreness. In addition, with 14 subjects, we had 79% to detect a significant difference in CK concentrations between massage and rest conditions using a two sided t-test and \(\alpha<0.05\) (76).
Chapter IV

RESULTS

Perceived Pain and Soreness

There was a significant main effect for DDS for pain (P=0.001), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Figure 4.1). However, there was no significant condition x time effect (P=0.218).

Figure 4.1: Descriptor Differential Scale (DDS) for Pain
In the massage and control conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significant main effect (P=0.001).
There was no significant main effect or condition x time effect for NPRS for pain or soreness (p>0.05), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Table 4.1). There was also no significant main effect or condition x time effect for DDS for soreness (P>0.05), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Table 4.1).

### Inflammatory and Immune Markers

There was a significant main effect for NC (P=0.012), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Figure 4.2). There was also a significant condition x time effect (P=0.036), however post-hoc analysis revealed no difference between conditions (p=0.410).

---

**Table 4.1:** Perceived pain and soreness for the Numerical Pain Rating Scale (NPRS), and perceived soreness for the Descriptor Differential Scale (DDS), for massage and control conditions, at baseline, 4 hours and 24 hours following onset of unaccustomed exercise.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean(SD)</th>
<th>4 hr Massage Mean(SD)</th>
<th>24 hr Massage Mean(SD)</th>
<th>Baseline-2 Mean(SD)</th>
<th>4 hr Control Mean(SD)</th>
<th>24 hr Control Mean(SD)</th>
<th>P-Value Main Effect</th>
<th>P-Value Condition* Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NPRS Pain (0-10)</strong></td>
<td>0.27(0.65)</td>
<td>1.18(1.45)</td>
<td>3.18(2.13)</td>
<td>0.18(0.41)</td>
<td>2.82(1.90)</td>
<td>4.32(2.46)</td>
<td>0.430</td>
<td>0.145</td>
</tr>
<tr>
<td><strong>NPRS Soreness (0-10)</strong></td>
<td>1.72(1.35)</td>
<td>2.22(1.50)</td>
<td>5.11(2.01)</td>
<td>0.83(1.17)</td>
<td>3.83(1.92)</td>
<td>7.22(1.64)</td>
<td>0.169</td>
<td>0.322</td>
</tr>
<tr>
<td><strong>DDS Soreness (0-17)</strong></td>
<td>5.21(2.53)</td>
<td>6.06(2.42)</td>
<td>7.31(1.30)</td>
<td>4.26(2.92)</td>
<td>6.62(3.08)</td>
<td>6.68(1.78)</td>
<td>0.638</td>
<td>0.499</td>
</tr>
</tbody>
</table>
Figure 4.2: Neutrophil concentrations (NC)
In the massage and control conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significant main effect (P=0.012).

There was a significant main effect for WBC (P=0.012), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Figure 4.3). There tended to be condition x time effect (P=0.055), but it did not reach statistical significance.
Figure 4.3: White Blood Cell concentrations (WBC)
In the massage and control conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significant main effect (P=0.012).

There was no significant main effect or condition x time effect for CK, IL-6, or CRP (p>0.05), adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Table 4.2).
Table 4.2: C-Reactive Protein (CRP), Creatine Kinase (CK), and Interleukin-6 (IL-6) concentrations for the massage and control conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significantly main effect. ^Significant condition x time interaction.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean(SD)</th>
<th>4 hr Massage Mean(SD)</th>
<th>24 hr Massage Mean(SD)</th>
<th>4 hr Control Mean(SD)</th>
<th>24 hr Control Mean(SD)</th>
<th>P-Value Main Effect</th>
<th>P-Value Condition* Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dl)</td>
<td>0.31(0.03)</td>
<td>0.59(0.97)</td>
<td>0.44(0.44)</td>
<td>0.44(0.50)</td>
<td>0.37(0.22)</td>
<td>0.434</td>
<td>0.335</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>184.15 (132.63)</td>
<td>231.00 (235.29)</td>
<td>340.08 (546.43)</td>
<td>162.54 (51.42)</td>
<td>224.39 (163.34)</td>
<td>0.664</td>
<td>0.661</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.50(1.73)</td>
<td>4.08(0.29)</td>
<td>4.33(0.78)</td>
<td>4.08(0.29)</td>
<td>4.75(1.76)</td>
<td>0.155</td>
<td>0.098</td>
</tr>
<tr>
<td>WBC</td>
<td>5.74(1.11)</td>
<td>8.52(2.53)</td>
<td>6.18(1.16)</td>
<td>7.85(1.77)</td>
<td>5.81(1.66)</td>
<td>0.012*</td>
<td>0.055</td>
</tr>
<tr>
<td>NC</td>
<td>3.07(1.05)</td>
<td>6.09(2.38)</td>
<td>3.65(0.88)</td>
<td>5.52(1.89)</td>
<td>3.49(1.53)</td>
<td>0.012*</td>
<td>0.036^</td>
</tr>
</tbody>
</table>

Table 4.2: C-Reactive Protein (CRP), Creatine Kinase (CK), and Interleukin-6 (IL-6) concentrations for the massage and control conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significantly main effect. ^Significant condition x time interaction.

Muscle Function

There was a significant main effect (p=0.002), adjusting for height, weight, BMI, 1RM bench, 1RM squat, and baseline. There was a significance condition x time (p=0.013) effect for ROM, where the massage condition showed significant decreases in ROM, at 24 hours, compared to control (p=0.006) (Figure 4.4).
**Figure 4.4: Range of Motion (ROM)**

In the massage (M) and control (C) conditions at baseline, 4 hours and 24 hours following onset of unaccustomed exercise. *Significant main effect (p=0.002).* ^Massage condition significantly lower than control (P=0.006).

![Graph showing range of motion over time](image)

**Table 4.3:** Range of motion (ROM) averaged over three trials per testing time point, for massage (M) and control (C) conditions, at baseline, 4 hours and 24 hours following following onset of unaccustomed exercise.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Mean(SD)</th>
<th>4 hr Massage Mean(SD)</th>
<th>24 hr Massage Mean(SD)</th>
<th>4 hr Control Mean(SD)</th>
<th>24 hr Control Mean(SD)</th>
<th>P-Value Main Effect</th>
<th>P-Value Condition* Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROM</td>
<td>91.25(7.97)</td>
<td>89.71(6.81)</td>
<td>89.24(5.09)</td>
<td>91.05(4.64)</td>
<td>89.65(7.91)</td>
<td>0.002</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Table 4.3:** Range of motion (ROM) averaged over three trials per testing time point, for massage (M) and control (C) conditions, at baseline, 4 hours and 24 hours following following onset of unaccustomed exercise.
Mood Assessment

There was no significant main effect or condition*time effect for POMS (p>0.05), after adjusting for height, weight, BMI, 1RM bench, and 1RM squat (Table 4.5).

<table>
<thead>
<tr>
<th>Total Mood Disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>POMS</strong> (TMD)</td>
</tr>
<tr>
<td>Baseline Mean(SD)</td>
</tr>
<tr>
<td>6.83 (19.25)</td>
</tr>
<tr>
<td>4 hr Massage Mean(SD)</td>
</tr>
<tr>
<td>-0.25 (15.75)</td>
</tr>
<tr>
<td>24 hr Massage Mean(SD)</td>
</tr>
<tr>
<td>-1.08 (13.53)</td>
</tr>
<tr>
<td>Baseline-2 Mean(SD)</td>
</tr>
<tr>
<td>5.83 (13.41)</td>
</tr>
<tr>
<td>4 hr Control Mean(SD)</td>
</tr>
<tr>
<td>2.92 (11.11)</td>
</tr>
<tr>
<td>24 hr Control Mean(SD)</td>
</tr>
<tr>
<td>8.92 (26.89)</td>
</tr>
<tr>
<td>P-Value Main Effect</td>
</tr>
<tr>
<td>0.969</td>
</tr>
<tr>
<td>P-Value Condition *Time</td>
</tr>
<tr>
<td>0.999</td>
</tr>
</tbody>
</table>

**Table 4.4:** Total mood disturbance (TMD) from the Profile of Moods State (POMS) questionnaire, for massage (M) and control (C) conditions, at baseline, 4 hours and 24 hours following onset of unaccustomed exercise.
Chapter V
DISCUSSION AND CONCLUSION

The goal of the current study was to investigate the effects of massage on perceived muscle soreness and pain, and inflammatory immune markers within the blood. The main findings, compared to control were massage; 1) significantly decreased perceived pain, 2) significantly increased WBC and NC count, and 3) significantly decreased muscle function. However, in contrast to our hypothesis, there were no differences between massage and control for perceived soreness, inflammatory markers (IL-6, CRP, and CK), and mood state. Taken together, these data indicate that massage is effective at dampening perceived pain and stimulating the inflammatory effect following unaccustomed exercise. However massage was not shown to be effective at decreasing perceived soreness, improving muscle function, or enhancing mood state.

Perceived Pain and Soreness

The current study found significant decrements in perceived pain following unaccustomed exercise, with no other significant findings for soreness. Perceived pain and soreness, as a result of DOMS, was assessed using the DDS and NPRS. Specifically, we found that massage significantly decreased pain at 4 hours but not 24 hours, but found no significant difference in soreness. These data are in contrast to several other studies (41, 76, 57). The discrepancy may be related to the delineation of pain and soreness. Most other studies measured only soreness (41, 76, 57), thus not separating pain from
soreness. For example, several studies found that massage significantly decreased soreness following exercise (35, 57, 76). In the current study, we separated out pain from soreness and found that massage decreased pain but not soreness.

Another possible explanation is that several studies used the Visual Analog Scale (VAS) for soreness (41, 52, 76, 57), whereas we used the NPRS and the DDS. The NPRS has been shown to be most responsive to slight changes when compared to the VAS, Verbal Rating Scale (VRS), and the Face Pain Scale-Revised (FPS-R) (25). Thus, we are confident that the methods used to assess pain and soreness were appropriate. Taken together, the results of the current study extend previous studies by showing that massage decreases pain, but not soreness. This may be advantageous to the athlete that completes multiple training bouts per day.

*Inflammatory and Immune Markers*

A novel finding of the current study is that both WBC and NC, were significantly higher after massage, compared to control. To our knowledge, studies have assessed the effects of massage on WBC and NC and exercise, both only reporting NC activity. Smith et al. (1994), found prolonged elevation of neutrophils with the massage group (76), whereas Hilbert et al. (2002), found no significant differences (35). This difference may hinge on time and duration of application of massage, with participants receiving an average of 25 min of localized massage, two hours post exercise for both previous studies (35, 76), where the current study applied 60 min full-body massage within 20 min following damaging exercise.
Additionally, the time of massage relative to exercise may influence immune markers. Previous studies have shown that exercise induced muscle damage results in membrane disruption and subsequent neutrophil infiltration (70, 80). In studies by Hilbert et al. (2002), and Smith et al. (1994), two hours may have been too long of interval for massage to affect inflammation (35, 76). The increases seen in the current study, along with prolonged levels in Smith et al. (1994), (76) could benefit the recovery process, as neutrophils have been shown to be the first WBC to enter damaged tissues and is required for the removal of damaged tissue (11). Nevertheless, the current findings indicate massage to significantly increase WBC and NC following unaccustomed exercise. Today, it is unclear whether an increase in WBC and NC, in response to massage, following exercise, is beneficial or not. The current study did not assess full recovery. Future studies should assess more time points and be of longer duration.

In contrast to our hypothesis, we found no significant differences in IL-6, CRP or CK concentrations with massage, compared to control, following unaccustomed exercise. While there did appear to be a trend for condition x time with IL-6 (p=0.098), it did not reach statistical significance. There is one other study that examined the affects of massage on IL-6 (17). Crane et al. (2012), found that massage attenuated IL-6 (17). These findings are not consistent with the current study. It is possible that the mode of exercise and type of specimen analyzed influenced the results. In the current study, serum IL-6 levels were assessed at baseline, 4 and 24 hours, with a full-body 60 min massage, following 90% max squat and bench press activity. In contrast, Crane et al. (2012), assessed IL-6 through muscle biopsy at baseline, 10 min., and 2.5 hours
following massage, after an exhaustive bout of aerobic activity, at 85% of peak VO2 (17). Taken together, these data indicates that further studies are needed to determine the effects of massage on IL-6 following exercise.

In the current study we found no effect of massage on CRP concentrations, which was surprising, given that CRP is a main inflammatory marker that is augmented after exercise (44, 47, 56). However, CRP was assessed in one other massage study (29), showing no effect of massage on CRP concentrations, following maximal eccentric activity (29). Our results are in line with this study, as Fuller et al. (2014), found no significant differences in CRP between massage and vibration therapy (29). Taken together, these data indicate that massage has no effect on CRP. However, with so few studies, further research needs to be conducted to explore the effects of massage on CRP following exercise.

Additionally, we found no effect of massage on CK concentrations. This is consistent with some, but not all previous studies (41, 58, 76, 88). Jakeman et al. (2010), found that massage had no impact on CK following 100 plyometric drop jumps (41). Likewise, Miller et al. (1996), found no massage effect on CK after eccentric knee extensions to fatigue (58). In contrast, Zainuddin et al. (2005), found significant decreases in CK concentrations with massage following 10 by 6 max eccentric actions of elbow flexors (88). Moreover, Smith et al. (1994), found reduced levels of CK in the massage group compared to the control, after completing 4-5 sets of 35 eccentric actions of the forearm flexors and extensors (76).
Differences may be related to mode of exercise. Mode of exercise has been shown to elicit varying responses of inflammation (36, 24, 48). For example, it appears that arm exercise following massage influences CK concentrations (76, 88), however, leg exercise does not (41, 58). Likewise, Hirose et al. (2004). found minor, but significant changes in cytokines and inflammatory mediators following repeated bouts of eccentric exercise, when compared to endurance exercise (36). These data taken together suggests massage has no effect on plasma CK levels at 4 hours or 24 hours following damaging physical activity.

**Range of Motion**

In contrast to our hypothesis, the current study showed massage to significantly decrease ROM over time and between groups at 24 hours following Unaccustomed exercise. The mechanism causing the further decrements in the massage group is likely due to the increased inflammatory response, as indicated by the increased levels of WBC and NC, resulting in increased swelling. Our results are in partial contrast with several other well controlled studies (35, 57), which similarly found ROM to decrease significantly over time (35, 57). However, in contrast to the current study, Hilbert et al. (2003), additionally found significant decreases at 48 hours following maximal eccentric activity, and both previous studies found no group interaction (35, 57). In complete contrast to the current study, one study found significant recovery in the massage group compared to the control (39). It is very likely that this effect is the result of time of application of intervention, as the current study applied massage post damaging activity,
and Imtiyaz et al. (2014), applied intervention prior to damaging activity (39). Taken together, these data suggest massage, following exercise, has an undetermined effect on ROM.

*Mood State*

While several previous studies have found massage to enhance mood (26, 34, 86), the current study found POMS not to be significantly affected by massage following unaccustomed exercise. Few relevant prior studies have assessed psychological state with massage following physical activity (34, 35). Our results are not in-line with Hemmings et al. (2000), which showed massage significantly increased perceived recovery (34). It is very likely that this discrepancy is related to the mode of exercise. The participants of the current study completed 6 sets of 8 reps at 90% of their max 1 RM, followed by a full-body recovery massage, where Hemmings et al. (2000), completed 5, 2 min. rounds of 80 boxing punches, followed by a 30 minute massage that targeted the muscles used in boxing (34). Nevertheless, our data suggests that massage has no effect on mood state following unaccustomed exercise. Our results were consistent with a previous, well controlled, exercise/massage study (35).

*Limitations*

Possible limitations included, reactive or interactive testing effects, and multiple treatment interference, due to the crossover design, and possible learning effect of exercise. Likewise, subjects and researchers alike, were not blinded to treatment, leading
to potential preconceived notions for outcome. This includes explanation of forms, treatments and procedures. Additionally, there was the possibility of experimental mortality/retention, with the deterrent of three needle blood draws, and time commitment of the subjects with six meetings over an eight week period. Further limitations included the limited population of college age, female students, from CalPoly University, San Luis Obispo, CA. Additionally, there were incomplete or missed NPRS and DDS scores for participants who chose not to complete form, and/or due to the confusing nature and hard to understand directions for DDS (19). Likewise, blood analysis of IL-6 and CRP, was not sensitive enough to detect the low-level changes in these variables, and assessment time points did not exceed 24 hours. There was also only one massage therapist that completed massage treatments, only one modality of massage treatment, and only one mode of exercise.

Summary and Future Directions

In summary, we hypothesized that massage after exercise, relative to no massage, would 1) decrease perceived muscle soreness and pain 2) decrease inflammatory immune markers (CK, IL-6, CRP, NC) and 3) increase range of motion (ROM). Partially confirming our hypothesis, we observed decreased perceived pain, and stimulation of WBC and neutrophil activity, with massage following unaccustomed exercise. Future massage studies following high intensity exercise need to confirm our results that massage stimulates the inflammatory immune response of WBC and NC. In addition, future studies need to look at the role of WBC and NC activity to determine if it
positively enhances the phagocyte and macrophage response to accelerate recovery, or if it negatively interferes, prolonging recovery and impeding athletic performance.

In contrast to our hypothesis, we noted no affect of massage on perceived soreness, inflammatory immune markers (IL-6, CRP, and CK), or mood state. Previous studies however, have shown localized massage to have some benefits following damaging exercise (17, 34, 35, 41, 57, 58, 76, 88). This may be directly related to the mode of exercise, as mode has been shown to induce varying responses to exercise (36, 24, 48). In the current study, we assessed perceived physical pain and soreness along with circulating concentrations of inflammatory markers, with massage following unaccustomed exercise. Future studies should directly compare the rate of recovery with massage following various modes of exercise, as the impact of massage following unaccustomed exercise still remains unclear. In general, massage seems to be more effective at decreasing perceived pain, which could be more psychological as opposed to physiological.

Therefore, assessing the impact of massage following exercise is necessary to determine its role in the inflammatory response. It is quite possible that massage following unaccustomed exercise may accelerate recovery. From a clinical perspective, being able to accelerate recovery following an introduction to, or a change in exercise, may have great value to the athlete's recovery and overall performance.
BIBLIOGRAPHY


37) Hiruma, E. (2005). Effects of massage and compression treatment on delayed onset muscle soreness following maximum calf-raise exercise: 1356 board #211 3:30 pm - 5:00 pm. *Medicine & Science in Sports & Exercise, 37*(Supplement), S265-S266.


A research project on massage and recovery from exercise is being conducted by graduate student, Courtney Crow, in the Department of Kinesiology at Cal Poly, San Luis Obispo, under the supervision of Dr. Todd Hagobian. The purpose of the study is to investigate whether massage has an effect on perceived physical soreness and perceived physical pain, range of motion, emotional wellbeing, and the inflammatory blood biomarkers, neutrophil count, interleukin-6, C-reactive protein, and creatine kinase, after high intensity exercise.

You are being asked to participate in this study, and will complete 2 separate conditions: high intensity resistance exercise and massage, and high intensity resistance exercise and passive rest. You will meet with the primary investigator five times over an eight week time period. In addition, four and twenty-four hours after each condition follow-up blood analysis will also occur. Please be aware that you are not required to participate in this research and you may discontinue your participation at any time without penalty. You may also choose not to participate in specific procedures or respond to any questions that you would prefer not to answer.

At the first meeting you will complete preliminary procedures, including a Physical Activity Readiness Questionnaire (PAR-Q) and Health History form, to assess your eligibility for the study. If you meet the criteria for the study, you will then complete a brief cycling warm-up followed by squat and bench-press activity. You should plan one and a half hours for this first session. Within the next four weeks, you will meet to complete testing procedures. After an overnight fast, we will meet at the CalPoly Health Center and a blood draw will be taken by a certified phlebotomist. Then your mood status, and perceived soreness and pain ratings will be measured using questionnaires. Then, we will walk over to the ASI Recreations Center to complete a range of motion test and the first exercise session. After a brief cycling warm-up, you will complete the squat and bench-press exercise. Within two hours after exercise, you will either rest (no massage) or have a 60-minute full body massage of the feet, legs, buttocks, back, arms, chest, neck, shoulders, and scalp. The female Massage Therapist, Courtney Crow, is California State Certified and has 11 years of experience. You should plan four and a half hours for this session. Four and forty-eight hours after the exercise, you will complete the questionnaires and have another blood draw by the CalPoly Health Center (you should plan twenty minutes for the subsequent blood draws). Thus the total time required to participate is approximately 12 hours. Please refrain from exercise and alcohol 24 hours prior to any and all exercise sessions. Please also note that your participation in this study will mean that you will not be allowed to use medications or ice, other than Arnica Montana cream, for pain relief prior to or within 24 hours following the exercise or massage.

The possible risks associated with participation in this study include all risks of exercise and use of equipment, including but not limited to exhaustion, probable sore muscles (you may use Arnica Montana cream to help attenuate muscle soreness), injury to muscles, ligaments, tendons, joints,
or other body parts, shortness of breath, nausea, abnormal blood pressure, fainting, dizziness, disorders of heart rhythm, and in very rare instances heart attack, stroke, or even death (1). The possible risks associated with massage include, but are not limited to muscle soreness, micro-tearing bruising, inflammation (2), headache, nausea, allergic reactions due to massage oil/lotion. In very rare cases, temporary nerve damage, and infectious skin conditions, have occurred (3). The possible risks associated with laboratory blood draws include, but are not limited to, redness, soreness, itching, lump, bruising, and in rare cases, infection at the needle injection site (4). If you should experience musculoskeletal injury, cardiac episode, shortness of breath, or any of the other associated risks listed above, please be aware that you may contact CalPoly Health Center, or Faculty Advisor, Todd Hagobian, Ph.D., at 805-756–7511, or Primary Investigator, Courtney Crow, at 714-873-6704.

Possible direct benefits of participation in this research include increased muscular strength and muscle tone. It is possible that massage will decrease muscle soreness and influence the inflammatory response of the body after exercise, which may improve (i.e. decrease) recovery time.

To protect your privacy, your answers to the questionnaires and exercise data collected will be kept confidential, and only seen by the Primary Investigator, Courtney Crow, and Faculty Advisor, Todd Hagobian. Each of you will be issued an identification number. That number will be used on all documentation. No names or personal information will be used. In addition, all documents and data will be stored in a locked file cabinet. Your name will not be used in any reports of this research without your permission.

If you have questions regarding this study or would like to be informed of the results when the study is completed, please feel free to contact the Primary Investigator, Courtney Crow at 714-873-6704. You may also contact their Faculty Advisor, Todd Hagobian, Ph.D., at 805-756–7511. If you have questions or concerns regarding the manner in which the study is conducted, you may contact Dr. Steve Davis, Chair of the Cal Poly Human Subjects Committee, at (805) 756-2754, sdavis@calpoly.edu, or Dr. Dean Wendt, Dean of Research, at (805) 756-1508, dwendt@calpoly.edu.

If you agree to voluntarily participate in this research project as described, please indicate your agreement by signing below. Please keep one copy of this form for your reference, and thank you for your participation in this research.

____________________________________  ________________
Signature of Volunteer        Date

____________________________________   ________________
Signature of Researcher     Date
APPENDIX B

HEALTH AND FITNESS HISTORY QUESTIONNAIRE

Subject #: __________________ Date: _______________ Age: _______

Height: ___________ Weight: ___________ BMI: _______

1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

   Yes No High Blood Pressure
   Yes No High Cholesterol or High Triglycerides
   Yes No Diabetes
   Yes No Hypoglycemia (low blood sugar)
   Yes No Asthma

2. Have you ever had a glucose tolerance test? Yes No
   If yes, what were the results?

3. Have you ever had a fasting blood sugar test? Yes No
   If yes, what were the results?

4. For women:
   - Are you on hormonal birth control (pill, patch, etc) or estrogen replacement? Describe in detail:

   - Is your cycle regular? Yes No
   - Do you know the phase of your current menstrual cycle? Yes No
   - What was the date of your last menstruation?
   - What is the date of your next expected menstrual cycle?
5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?  
   Yes         No  
   If Yes, Please explain  

6. Are you currently taking any medications, including over-the-counter drugs such as aspirin, Tylenol or Ibuprofen? Please list: 

7. Do you smoke or use smokeless tobacco?  
   Yes         No  

8. Do you drink coffee or other caffeinated beverages?  
   Yes         No  
   What kind, how much and how often?  

9. Please list all vitamins, minerals and herbs and other nutritional supplements you’re taking: 

10. How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe: 

11. Do you exercise regularly?  
    Yes         No  
    What kinds of exercise?  
    How often?  
    Are squats and bench press apart of your regular exercise?  
    Yes         No  
    If yes, how many sets and reps at what weight increments?  
    Please describe how much walking you do on a daily basis:
12. How does your current exercise and physical activity compare to 6 months ago?

1 year ago?

13. Have you had a physical exam in the past two years?  
Yes    No

Please describe your assessment of your overall health:

14. Do you have any shrapnel, metal objects (plates, rods, screws, etc.), or electronic devices implanted in your body?  
Yes    No

If yes, please describe:

15. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain.

16. Have you injured yourself in the past 6 months?  
Yes    No

If Yes, Please explain

17. Do you have any open wounds?  
Yes    No

If Yes, Please explain

18. Do you have any joint conditions that would limit your ability to participate in intense physical activity?  
Yes    No

If Yes, Please explain

20. Have you ever received a professional massage?  
Yes    No
21. Do you receive massage on a regular basis? Yes  No
If yes, how often

To the best of my knowledge, the above information is true.

Participant's Signature: ___________________________ Date: ______________

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APPENDIX C

Profile of Mood States Questionnaire

Below is a list of words that describe feelings people have. Please read each word carefully and circle the number that best describes how you have been feeling over the past hour. [PLEASE CIRCLE ONLY ONE ANSWER FOR EACH QUESTION.]

<table>
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<tr>
<th>Word</th>
<th>0 = Not at all</th>
<th>1 = A little</th>
<th>2 = moderately</th>
<th>3 = Quite a bit</th>
<th>4 = extremely</th>
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0–10 Numeric Pain Rating Scale

No pain | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Worst possible pain

Moderate pain
Descriptor Differential Scale

EACH WORD REPRESENTS AN AMOUNT OF SENSATION. RATE YOUR SENSATION IN RELATION TO EACH WORD WITH A CHECK MARK.