

**Mirthful laughter and muscle soreness/pain**

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## **Study Protocol**

### **Participants**

Forty adults (15 men and 25 women) 20-40 years of age were recruited. Participants were included if they were apparently healthy, normotensive, and free of musculoskeletal disease. Participants were excluded if they had known cardiovascular or musculoskeletal disease or if their pain tolerance was not reachable in a blunt force test on the quadriceps (maximal pressing force allowed by the IRB was 245 N). Participants were instructed to keep their normal daily routine including caffeine consumption throughout the study period to prevent the effects of lifestyle factors on pain tolerance measurements. The Institutional Review Board approved the study, and a written informed consent was obtained from all participants. The study was registered at the National Institutes of Health ([ClinicalTrials.gov](https://clinicaltrials.gov)).

### **Preliminary study to evaluate various pain tolerance tests**

Hemodynamic factors are known to influence pain perceptions and pain responses (Bruehl & Chung, 2004) as the brain regions regulating the cardiovascular system are known to overlap substantially with those that contribute to anti-nociception (Randich & Maixner, 1984). In order to select the optimum pain tolerance protocol that would not evoke substantial hemodynamic responses, changes in blood pressure and heart rate in response to 3 commonly-used pain tolerance tests (blood pressure cuff occlusion, blunt force application on muscles, and cold pressor test) were compared. Participants were instructed to lie supine with a beat-by-beat blood pressure monitor (Portapres, Finapres Medical Systems, Enschede, Netherlands) that was placed on the middle phalanx of the middle finger on the left hand. The Portapres was connected to the WinDaq data acquisition software (DATAQ Instruments, Akron, OH) to record heart rate and blood pressure for later analysis.

A load cell (Transducer Techniques, Temecula, CA) attached to a pan head screw and metal handle was used to determine the load placed on the quadriceps of each participant. The force transducer applied blunt force and measured the force by running through an amplifier (TMO-2, Transducer Techniques, Temecula, CA) connected to our data acquisition software (WinDaq). Blunt force was steadily increased until the participant indicated that the pain was not tolerable, at which point the test was terminated. The blunt force was applied half way between the anterior superior iliac spine and the top of the patella. The specific spot was marked in order to avoid testing directly on the same exact spot that could inflict residual tenderness. The rate of increases in applied force was controlled through feedback on the WinDaq data acquisition system.

For the blood pressure cuff pain test, a standard blood pressure cuff was placed on the right upper arm. The cuff was gradually inflated at 15 mmHg per second until the participant indicated that they were at the limit of their pain tolerance. Per the IRB guideline, the maximum blood pressure was set at 300 mmHg. The cold pressor test was performed with the participants submerging their hand up to their wrist in ice water of about 4 °C. Participants were told to remove hand when they reached their pain tolerance.

### **Main experimental protocol**

The main study was conducted using a randomized controlled cross-over approach. Participants reported to the laboratory a total of seven times. Participants underwent a preliminary testing session followed by three visits each for the two video watching interventions. The interventions were separated by a minimum of one week as a washout. Each intervention included one day of inducing muscle soreness in one leg through eccentric muscle contractions (see below), a second day of testing muscle soreness and pain tolerance and watching a 30-minute video (either a comedy or documentary), and a third day of testing muscle soreness and pain tolerance again to see if the effects of the video viewing persisted the next day (i.e., twenty-four hours after the video viewing).

The second intervention was identical to the first intervention except that muscle soreness was induced on the contralateral leg and the video was switched to either comedy or documentary. The orders of the videos and legs were randomized by a research assistant flipping a coin, and participants watched a randomly-assigned video. If the video was a comedy, the participants picked stand-up comedians of their choice in order to meet with their individual preference of comedy/humor. The video was watched for 30 minutes with two other people in

the room to make it a social setting to induce greater degree of laughter. When the video was a documentary, an uninteresting serious documentary was chosen by the investigators.

### **Eccentric exercise**

Participants began with a five-minute warm-up at 50 W on a cycle ergometer (Monarch Exercise, Vansbro, Sweden). Participants were then secured to the isokinetic dynamometer chair in a seated position with chest and waist straps (Biodex System 2, Shirley, NY). The movement began at a speed of 45 degrees/second at a knee angle of 90 degrees. The participants were instructed to resist maximally until full flexion was reached and completed 12 sets of 10 repetitions of eccentric maximal voluntary actions. Participants aimed for 70% or better of the previously-tested one repetition maximum for the eccentric phase. Exercise sets were separated by one minute of rest. This protocol has been utilized successfully in previous studies to elicit delayed onset muscle soreness (Armstrong, Warren, & Warren, 1991; Prou, Guevel, Benezet, & Marini, 1999; Vassilis et al., 2008).

### **Body composition**

The seven-site skinfold assessment was utilized to estimate body fat percentage using Lange skinfold calipers (Cambridge Scientific, Cambridge, MA). Body fat level was collected primarily as a descriptive variable but could potentially contribute to pain tolerance due to the association between body fat and inflammatory mediators (Gustafson, 2010) that could sensitize nociceptors to pain (Marchand, 2008).

### **Muscle strength**

A reduction in muscle strength is an objective indication of muscle soreness (Byrnes & Clarkson, 1986). Knee extensors of both legs were tested for maximum strength using the isokinetic dynamometer (Biodex System 2, Shirley, NY). Both the concentric and eccentric

phases of the strength test were set at a speed of 60 degrees/second. Subsequently, isometric (static) muscle strength tests were performed. The lever was locked at 60 degrees and then at 90 degrees, and the participant was instructed to try to extend the leg and hold it for 5 seconds.

### **Pain**

The visual analog scale pain score (VAS) is an established instrument for the assessment of subjective pain (Jensen, Karoly, & Braver, 1986) that has been found to be reliable (Price, McGrath, Rafii, & Buckingham, 1983). It is a scale from 0 to 10 showing faces and descriptions of what participants may feel (Jensen et al., 1986). Participants picked the number (i.e., pain score) that corresponded best to how their sore leg felt. Participants also had their pain tolerance measured using the force test as an objective measure.

### **Mood and psychological state**

The Coping Humor Scale (CHS) and the Situational Humor Response Questionnaire (SHRQ) were administered to all the subjects (Martin, 1996). The Positive and negative affect schedule (PANAS) was administered before and after watching the video to evaluate changes in mood state. High negative affect is characterized by subjective distress and unpleasurable engagement, and low negative affect by the absence of these feelings. Positive affect represents the extent to which an individual experiences pleasurable engagement with the environment (Watson, Clark, & Tellegen, 1988).

### **Laughter**

In order to quantify the amount of laughter, a Pneumotrace II respiration transducer (UFI, Morro Bay, CA) was worn above the umbilical region while watching the videos. The respiration transducer was attached to the WinDaq data acquisition system (DATAQ Instruments, Akron,

OH) to record the data. The irregular respiratory pattern was taken as indications of laughter as previously described (Bloch, Lemeignan, & Aguilera-T, 1991; Svebak, 1975).

### **Statistical analyses**

Descriptive characteristics were expressed as means $\pm$ SEM. Paired t-tests were conducted between the various outcomes of the comedy and documentary treatments with the probability level set at  $p < 0.05$ . To compare physiological changes caused by each pain tolerance test, linear mixed models were used. A random intercept was included for each participant since the experiment was repeated measures. ANOVA tables for each fitted model were constructed. In the case of significance, multiple comparisons were performed using the Bonferroni method. To control for important covariates and to investigate significant predictors of pain tolerance, linear mixed model analyses that include fixed and random effects were used. The linear mixed model included fixed and random effects, to predict the pain tolerance. The fixed component described the sample's relationship between the covariates, predictors, and pain tolerance while the random component described the variation within each participant. The inclusion of a random intercept for each participant in the mixed model was justified because of the large variance in pain tolerance. Predictor variables included leg, time, and treatment, while covariates included sex, SHRQ, CHS, PANAS scores, and body fat percentage. A simple ordinary least squares regression was used to investigate if a relationship exists between the time spent laughing and the change in pain tolerance. To determine the total variability in pain tolerance, the intraclass correlation coefficient (ICC) was calculated.