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Intramuscular Temperature of Rectus Femoris During Cold Water
Immersion

By

Nicholas Rech

A Plan B project submitted in partial fulfillment
of the requirements for the degree

Of

MASTER OF SCIENCE

In

HEALTH AND HUMAN MOVEMENT

Approved:

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Committee Member

Utah State University
Logan, UT
2013

ABSTRACT

Purpose: To establish a protocol for cold water immersion (CWI) temperature and duration based on adipose tissue thickness and desired cooling at 2 cm sub-adipose tissue of rectus femoris (RF) after exercise.

Methods: Sixteen participants received a CWI treatment (10 °C) until either intramuscular thigh temperature (2 cm sub-adipose) decreased 7 °C below pre-exercise level or 30 minutes was reached. Temperature was recorded every 30 seconds using skin and implantable fine-wire thermocouples. Temperature reductions and cooling rates were analyzed.

Results: Five out of the 16 participants cooled 7 °C below baseline within 30 min. Adipose thickness and percent body fat are significantly correlated with CWI cooling rate ($M=0.27$ °C/min) ($p<.01$). Intramuscular tissue continues to significantly cool post-CWI ($p<.005$) an average of 1.7°C for an average of 23 min. Minimal intramuscular tissue cooling is achieved after 10 and 15 min ($M=2.5^{\circ}\text{C}$, $M=4.0^{\circ}\text{C}$ respectively).

Discussion: CWI treatment protocols need to be based on adipose thickness and target tissue depth. Recommended cooling of acute injuries to reduce secondary hypoxia is likely greater than cooling needed for post-workout recovery to reduce EIMD.

INTRODUCTION

Cryotherapy, specifically cold water immersion (CWI), is the therapeutic use of cold. It is a widely used modality used to treat exercise induced muscle damage (EIMD) (Ingram, Dawson, Goodman, Wallman, & Beilby, 2009; Jakeman, Macrae, & Easton, 2009; King & Duffield, 2009). Damage includes disruption of the sarcolemma, fragmentation of the sarcoplasmic reticulum, lesions of the plasma membrane, cytoskeletal damage and swollen mitochondria (Eston & Peters, 1999). CWI is used to reduce tissue temperature. The reduction in tissue temperature decreases swelling by slowing blood flow to the affected area (Cochrane, 2004; Wilcock, Cronin, & Hing, 2006; Bailey et al., 2007; Jakeman et al., 2009),

pain by slowing nerve conduction velocity (Goodall & Howatson, 2008; Bleakley & Hopkins, 2010; Eston & Peters, 1999; Vaile, Halson, Gill, & Dawson, 2008) and secondary cell damage by suppressing cellular metabolism (Yanagisawa, Niitsu, Takahashi, Goto, & Itai, 2003; Bender et al., 2005; Bleakley & Hopkins, 2010; Wilcock et al., 2006; Rupp, Selkow, et al. 2012). Increasingly, CWI has gained an overwhelming amount of anecdotal support as well as empirical evidence for the treatment of indices of EIMD: creatine kinase, myoglobin, pain, soreness, fatigue, strength loss, decreased range of motion, decreased speed and edema (Eston & Peters, 1999; Kinugasa & Kilding, 2009; Yanagisawa et al., 2003; Rowsell, Coutts, Reaburn, & Hill-Hass, 2009). Given the potential detrimental impact that intense training and competition can have on athletic well-being, effective post-exercise recovery procedures are vital for optimal performance (Ingram et al., 2009). Therefore, CWI is employed to enhance recovery and hasten return to optimal performance capabilities (Cochrane, 2004).

Unfortunately, the present research is inconsistent in the results CWI has on recovery after various types of muscle damaging exercise (Rupp, Herman, Hertel, & Saliba, 2012). No study to date has been able to unequivocally determine that CWI improves the various indices associated with EIMD (Kinugasa & Kilding, 2009; King & Duffield, 2009).

Possibilities for these discrepancies potentially lay in the methodological inconsistencies in cold temperature, frequency and immersion duration (Wilcock et al., 2006; Goodall & Howatson, 2008; Bleakley & Hopkins, 2010; Cochrane, 2004; Ingram et al., 2009; Jakeman et al., 2009; Rupp, Selkow, et al. 2012). No specific water temperature range has been determined for cryotherapy (Wilcock et al., 2006). Interestingly, a number of studies provided no reference or explanation for their parameters of CWI (Yanagisawa et al., 2003; Barcroft & Edholm, 1946; King & Duffield, 2009; Lane & Wenger, 2004; Rowsell et al., 2009). Jakeman, Macrae, and Easton (2009) and Vaile, Halson, Gill, and Dawson (2008) noted the CWI protocols used in their studies were based on parameters similar to what is used in the field but listed no scientific reasoning for the parameters of their protocols. This thinking of doing what has always been done without seeking scientific backing may be a factor contributing to the variability of results.

Meeusen and Lievens (1986) recommend that ice pack application for 10-15 minutes is sufficient to reduce intramuscular temperature 7-10°C at a depth of 1cm. This critical 10°C temperature decrease must be achieved for cellular metabolism to slow by approximately 2 fold (Bleakley & Hopkins, 2010). This data was referenced by Bailey et al. (2007), Eston and Peters (1999) and Goodall and Howatson (2008) as the source of their selected CWI parameters.

Multiple studies used CWI protocols that were under the recommended 10 minutes or immersion was not continuous but rather alternated between CWI and resting out of the water (Rowse et al., 2009; Ingram et al., 2009; King & Duffield, 2009). Therefore, the protocols were likely insufficient to cause adequate tissue cooling. Many studies looking at the efficacy of CWI have used data from ice bag applications as their guide for selecting parameters. Merrick, Jutte, and Smith (2003) in a well controlled study, using intramuscular thermocouples at the surface, 1cm and 2cm sub-adipose tissue, examined tissue cooling using ice bags. After 15 minutes surface temperature decreased approximately 22°C while temperature at 1cm decreased approximately 6°C and 2cm approximately 2°C, which is well above what is cited by Meeusen and Lievens (1986). Yanagisawa, Niitsu, Takahashi, Goto, and Itai (2003) probably showed the best results (tendency to reduce soreness and reduced edema of the calf) using a 15min CWI protocol at 5°C. These results may not be generalizable to larger body parts such as the thigh musculature. This may be further evidence of the inadequacy of current CWI parameters of the thigh. There is a lack of data specifying adequate CWI temperature and duration on intramuscular cooling, which may be the reason that there is so much inconsistency between CWI studies (Rupp, Herman, et al. 2012).

Intramuscular temperature of the calf was shown to decrease in a similar fashion using CWI, at 12°C, compared to crushed ice bag (Rupp, Herman, et al. 2012). However, it took an average of 40 minutes for the temperature 1cm sub-adipose tissue to decrease 7°C. The time to the target cooling temperature is much longer than current treatments and data gathered from the calf may not be a good source for determining treatment parameters on the thigh due to multiple factors affecting heat transfer.

The transfer of heat from one body to another depends on several factors. These factors include temperature gradient, relative mass of the bodies, size of contact area, treatment duration, depth of desired

cooling, the heat capacity of each material, metabolic activity and perfusion (Merrick, Jutte, & Smith, 2003; W. J. Myrer, Myrer, Measom, Fellingham, & Evers, 2001). With this in mind no study has compared limb size to tissue cooling. Deep tissues are cooled by losing heat to more superficial tissues which have been cooled (Merrick et al., 2003). Since a larger limb would have more tissue, the warm tissue below the cooling tissue may re-warm the cooling tissue faster than it can cool in a reasonable treatment duration. CWI has a lower ability to absorb heat than ice bags since it does not undergo a phase change (Merrick et al., 2003) but it contacts a larger area.

In addition, few studies using CWI have based their treatment parameters on amount of adipose tissue present over treatment area (Long, Cordova, Brucker, Demchak, & Stone, 2005). There are clear trends that the magnitude of temperature reduction is smaller as skinfold increases (Bleakley & Hopkins, 2010; Otte, Merrick, Ingersoll, & Cordova, 2002; Stocks, Taylor, Tipton, & Greenleaf, 2004). Therefore, depth of adipose tissue should be taken into account. It would not be adequate to suggest the same CWI protocol for individuals of significantly different adipose thicknesses. Otte, Merrick, Ingersoll, and Cordova (2002) found that skinfold thickness played a large role in the ability of an ice bag to drop the intramuscular temperature 1cm sub-adipose tissue by the 7°C recommended by Meeusen and Lievens (1986). He found that a skinfold under 10mm takes 9min to cool, 11-20mm takes 23min, 21-30mm takes 40min and 31-40mm takes 60min. W. J. Myrer, Myrer, Measom, Fellingham, and Evers (2001) also agreed that subcutaneous adipose tissue is a significant factor in the magnitude and rate of intramuscular tissue cooling at 1 and 3cm sub-adipose tissue. Rupp, Herman, Hertel, and Saliba (2012) found no significant correlation between time to decrease intramuscular temperature and adipose tissue thickness. However, they do discuss that the majority of the participants had adipose thickness between 11-20mm which was not a large enough range to show a difference.

Gender differences may be another variable uncontrolled for that is contributing to inconsistent results. Stocks, Taylor, Tipton, and Greenleaf (2004) listed several differences in women such as surface area to mass ratio, skin blood flow, adipose distribution and menstrual rhythm that could necessitate different CWI protocols for women than men. Some CWI studies used women (Eston & Peters, 1999;

Jakeman et al., 2009; King & Duffield, 2009), men (Bailey et al., 2007; Rowsell et al., 2009; Yanagisawa et al., 2003; Ingram et al., 2009; Lane & Wenger, 2004; Vaile et al., 2008) or a combination (Rupp, Selkow, et al. 2012). It may not be efficacious to compare research using different populations until how CWI affects the intramuscular environment of each gender has been explicitly determined.

No study to date has determined the percentage of muscle that is adequately cooled by CWI. Even for studies that show adequate cooling to 2cm sub-adipose tissue (Long et al., 2005), is that a majority of the target muscle? The relevant temperature reductions must occur within the injured tissue. This being the muscle layer at and around the point of injury (Bleakley & Hopkins, 2010). CWI of the forearm in 12°C water cooled the brachioradialis muscle by 10°C 1in below skin level (Barcroft & Edholm, 1946). This same degree of cooling may not be similar in the thigh due to its larger mass. A possible reason for the lack of empirical evidence to support CWI to reduce indices of EIMD is that not enough of the damaged muscle is being cooled.

Beginning tissue temperature is another variable that needs to be accounted for. Prior exercise shortens the time it takes to cool intramuscular tissue at 1cm and 2cm sub-adipose tissue using ice bags. This is because the muscle tissue is warmer creating a larger temperature gradient which allows a greater amount of heat transfer (Long et al., 2005; Bender et al., 2005). Tissues at a 1cm depth cool faster than at a 2cm depth (Long et al., 2005; Yanagisawa, Homma, Okuwaki, Shima, & Takahashi, 2007). This is because the cooling effect of the cold treatment will have to penetrate more tissue thus attenuating the temperature gradient. Instead of being cooled by the cold modality directly, deeper tissues are cooled by the slightly cooler tissues superficial to them. This is a much smaller temperature gradient and the gradient continues to decrease as deeper tissues are reached (Merrick et al., 2003; Bleakley & Hopkins, 2010; Kennet, Hardaker, Hobbs, & Selfe, 2007).

There is a critical need for a standardized approach for the design of CWI protocols. Currently, too many variables exist between literature studying the effects of CWI on EIMD. A standardized treatment approach will lead to more focused research producing unequivocal results of the efficacy of CWI.

PURPOSE

To establish a standard for CWI temperature and duration based on adipose tissue thickness and desired cooling at 2cm sub-adipose tissue of the rectus femoris (RF) after exercise. Secondary purpose is to determine percentage of total thigh musculature adequately cooled.

SUBJECTS

Sixteen participants (age = 24.3 ± 1.84 years, height = 176.4 ± 12.7 cm, mass = 86.6 ± 29.4 kg, anterior thigh adipose thickness = 1.1 ± 0.37 cm, thigh girth = 57.3 ± 5.4 cm, percent body fat = 22.0 ± 7.5), tissue depth to bone = 5.0 ± 0.7 cm, thigh volume 9655 ± 2522 cm³) were recruited from the university graduate student population. Participants of varying thigh skinfold thickness were used. According to Otte et al. (2002) there are significant differences between the amount of cooling between adipose thickness of 0-10mm, 11-20mm and 21-30mm. Therefore, subjects falling into each category were used to obtain data for the entire range. Each participant gave written informed consent before participating in the study.

INSTRUMENTATION

Intramuscular temperature was measured with an IT-18 implantable thermocouple (Physitemp Instruments Inc, Clifton, NJ) inserted through a 18 gauge x 8.89cm catheter (PSS World Medical, Jacksonville, FL). Surface temperature was measured with an IT-18 thermocouple as well. The thermocouples will be connected to a 8 channel Thermes USB portable temperature data-acquisition device (Physitemp Instruments Inc, Clifton, NJ). The manufacturer indicated temperature measurement setup is noted to be accurate to within $\pm 0.2^{\circ}\text{C}$. Adipose tissue thickness and muscle thickness were measured using a BodyMetrix ultrasound device (InteleMetrix, Livermore, CA). A stationary cycle ergometer (Freemotion Fitness, Colorado Springs, CO) was used for the exercise bout.

PRODEDURE

Subjects were familiarized with all procedures prior to testing. Each subject reported to the Worley Sports Medicine Research Center having refrained from consuming alcohol, caffeine, or food for 1 hour and from any vigorous activities for 2 hours before the test to help stabilize extremity blood flow. Volume of the thigh was calculated by measuring thigh circumference at the midpoint between the greater trochanter and 1 cm superior to patella. Thigh volume will be calculated as follows:

- Total area of slice, $A_T = C_T^2/4\pi$, where C_T is the leg circumference or girth.
- Circumference of inner circle (lean or muscle + bone girth), $C_L = C_T - \pi * T$, where T is the thickness of the superficial adipose tissue.
- Area of inner circle (lean tissue), $A_F = (C_L - 2\pi * T)^2/4\pi$
- Area of superficial adipose tissue (fat tissue), $A_F = C_T * T - \pi * T^2$.

Calculation of the lean or muscle + bone volume (VL), adipose volume (VF) and total volume (VT) of a limb segment assumed to be a truncated cone is given by the formulas:

$$V_L = A_L * h, V_F = A_F * h, V_T = V_L + V_F$$

where h is the height of the thigh measured from the greater trochanter to 1 cm superior to the patella (Tothill & Stewart, 2002).

With each participant standing, body composition was determined using a BodyMetrix A mode ultrasound device. Body composition was calculated using the Jackson and Pollack three site measurement. Thigh height was measured from 1 cm superior to the patella to the greater trochanter, Thigh circumference was taken at the midpoint between 1 cm superior to the patella and the greater trochanter. The midpoint was marked on the anterior thigh to indicate location of insertion of the temperature probe. An ultrasound scan was used to form a picture showing the

adipose-muscle and muscle-bone interfaces. From this scan depth of adipose tissue and anterior thigh muscle thickness was measured. Ultrasound gel at each measurement site was removed with a paper towel.

Thereafter, a 10x10cm area surrounding the midpoint mark was cleaned with povidone-iodine swabs. Three swabs were used in a circular pattern on each participant. The insertion site, 1 cm superior to midpoint mark, was anesthetized using 1 ml of Bupivacaine (Hospira, Inc., Lake Forest, IL).

The tube surrounding the probe insertion needle was cut so as to act as a stop when the needle reached a depth of 2cm sub-adipose tissue. A 4in spinal needle was inserted into the muscle 1 cm superior to the midpoint mark. Once at the correct depth the thermocouple was fed through the needle to the required depth. Pressure was maintained on the thermocouple wire as the needle was removed from the thigh to ensure that it stayed at the correct depth. When the needle was removed the wire was taped to the thigh using Cover Roll and the needle taped to the wire probe at a site proximal to the data acquisition device. The surface thermocouple was attached at a site 1 cm inferior to the midpoint mark. Waterproofing with a 8 x 8 cm strip of waterproof, transparent, adhesive film (OpSite™, Smith and Nephew, Largo, FL). The edges of the OpSite were anchored down with strips of adhesive stretch tape (Cover-Roll® stretch, BSN MedicalGmbH, Hamburg, Germany).

The leads were secured to the participants using the adhesive stretch tape just superior to the iliac crest to prevent the leads from being disturbed. The thermocouples were connected to the data-acquisition device and temperature recordings occurred every 30 seconds for the duration of the study.

Since the purpose of this study is to determine tissue cooling after exercise and it has been shown that tissues at different temperatures cool at different rates (Long et al., 2005), this study had participants perform an exercise bout to raise tissue temperature to simulate post work out conditions. The exercise condition consisted of 30 minutes of riding a stationary cycle ergometer while maintaining a heart rate between 130 and 150 beats per minute. Heart rate measurements were made routinely throughout the duration of exercise using a pulse oximeter (Nellcor Puritan Bennett Inc., Pleasanton, CA) to ensure constant levels.

The treatment consisted of immersion in cold water (10°C) up to the level of the iliac crest. Water temperature at 10°C was used because it is in the mid-range of CWI temperatures used in the literature and it is within the recommended range listed in sports medicine texts (Starkey, 2004; Jakeman et al., 2009; Lane et al., 2004; Yanagisawa et al., 2003; Vaile et al., 2008). Temperature recordings continued until both intramuscular and surface temperatures are 7°C less than their respective pre-exercise values or until 30 minutes passed. A decrease in tissue temperature of 7-10°C is needed to decrease tissue metabolism by 50% (Meeusen & Lievens, 1986). The reduction in tissue metabolism accompanied by a 7°C decrease in temperature was the reason 7°C was chosen as an adequate tissue cooling.

At the end of the immersion treatment, the participants dried off and stood. Intramuscular tissue temperature continued to decrease in all subjects. When the intramuscular tissue began to rewarm, recording was continued for 15 minutes and then stopped. Subjects stood after the CWI treatment rather than sat because standing will more closely simulate what an athlete normally does after treatment.

At the end of data collection the thermocouples were withdrawn and insertion depth remeasured to check if the thermocouple remained at the correct depth. The insertion site was

cleansed with an alcohol wipe and a bandage was applied. Needles were disposed of in appropriate sharps containers. The thermocouples were sterilized by an autoclave after each use. Sterile techniques were used during all invasive procedures

STATISTICAL ANALYSIS

Required sample size was calculated using G*Power version 3.1. An a priori repeated measures, between factors design with desire power (1-b) set at .80, a large effect size of .8, and alpha of .05 yielded a total sample size of 16 participants. SPSS was used to perform all statistics. Repeated measures ANOVA was used to determine interaction of temperature over time and interaction of adipose tissue with temperature change over time. Correlations were performed between independent and dependent variables. Cooled volume of muscle will be divided by total thigh muscle volume to determine percentage of total muscle cooled. Cooled volume was calculated by the following equations:

$r_W = r - c_D$, where r_W is the radius of warm tissue, r is total radius of thigh, and c_D is the cooling depth (2cm+adipose thickness).

$A_W = \pi * r_W^2$, where A_W is the warm tissue area.

$V_W = A_W * h$, where V_W is the volume of warm tissue and h is thigh height.

$V_C = V_T - V_W$, where V_C is cooled volume and V_T is total muscle volume.

% muscle cooled= V_C/V_T .

RESULTS

Five of the 16 participants cooled at least 7°C below the previously recommended baseline temperature while in CWI. Table 1 presents data collected from the participants prior to testing.

Table 1. Participant Thigh Measurements

	Adipose Thickness (cm)	Body Fat (%)	Thigh Circumference (cm)	Depth to Bone (cm)	Thigh Volume (cm ³)
Mean	1.1	22.0	57.25	5.0	9655
SD	±0.4	±7.5	±5.4	±0.7	±2522
Range	0.5-1.7	8.0-36.1	49.5-68.0	3.8-6.2	6332-14083

The subset that reached adequate cooling had a lower average adipose thickness (0.8 cm) than the group that did not (1.2 cm). Percentage of muscle cooled was $35.3 \pm 4.3\%$. Significant correlations were observed between percent body fat and CWI cooling rate [$r(16) = -.794, p < .01$], percent body fat and cooling rate post CWI [$r(16) = -.532, p < .05$], percent body fat and degrees cooled in CWI [$r(16) = -.753, p < .01$], percent body fat and degrees cooled post CWI [$r(16) = .695, p < .01$], percent body fat and time in CWI [$r(16) = .671, p < .01$], and percent body fat and post CWI cooling time [$r(16) = .771, p < .01$].

There were also significant correlations between adipose thickness and CWI cooling rate [$r(16) = -.789, p < .01$], adipose thickness and cooling rate post CWI [$r(16) = -.575, p < .05$], adipose thickness and degrees cooled in CWI [$r(16) = -.734, p < .01$], adipose thickness and degrees cooled post CWI [$r(16) = .678, p < .01$], adipose thickness and time in CWI [$r(16) = .670, p < .01$] and adipose thickness and post CWI cooling time [$r(16) = .787, p < .01$].

Significant correlations were also observed between thigh circumference and cooling rate post CWI [$r(16) = -.682, p < .01$], tissue depth to bone and cooling rate post CWI [$r(16) = -.535, p < .05$] and thigh volume and cooling rate post CWI [$r(16) = -.556, p < .05$]. Repeated measures ANOVA revealed a significant interaction between intramuscular temperature at each time measurement and adipose tissue thickness. ($F(1.352, 18.915) = 10.443, p < 0.005$) as seen in table 2. There was a significant main effect between intramuscular temperature at pre-bike and

post-bike ($p=.005$), post-bike and post-CWI ($p=.005$), post-CWI and lowest temperature ($p=.005$) and lowest temperature and end temperature ($p=.005$). Repeated measures ANOVA also revealed a significant interaction between the intramuscular cooling conditions in table 5 ($F(1.552, 23.276) = 109.437, p < 0.0005$).

Intramuscular CWI cooling rate calculated for each participant was used to make a prediction for time required to cool intramuscular tissue 7°C ($44.5 \pm 42.6^{\circ}\text{C}$). A prediction for cooling time was made using the equation of the trendline ($y = -0.3796x + 0.6791$) from the correlation between intramuscular CWI cooling rate and adipose thickness. Adipose thickness was substituted for the x variable and y equals the predicted cooling rate. 7°C was divided by this cooling rate to equal the predicted time to cool intramuscular temperature 7°C using the trendline equation ($46.2 \pm 54.6^{\circ}\text{C}$). An independent t-test revealed no significant difference between the means of the two predictions ($t(30) = -0.097, p = .92$). The prediction by the trendline equation was very accurate until adipose thickness reached 1.3 cm. At this point it began to break down which explains the larger standard deviation.

The CWI cooling rate was significantly greater than the post-CWI cooling rate ($t(30) = 2.77, p = .009$) as seen in table 4. Greater tissue cooling was achieved during CWI than post-CWI ($t(30) = 5.96, p = .000001$). A significant difference was not observed between CWI cooling time and post-CWI cooling time ($t(30) = .819, p = .419$).

Table 2 and table 3 show intramuscular and skin temperature respectively at each of the time points during the study. Table 4 and table 5 present intramuscular cooling measurements. Figure 1 and figure 2 show individual intramuscular and skin temperatures over time respectively. Figures 3 and 4 present highly significant correlations of intramuscular cooling rate

in CWI with adipose thickness and percent body fat respectively. Figures 5 and 6 present less significant correlations of intramuscular cooling rate post-CWI with adipose thickness and percent body fat respectively. Figures 7 and 8 present significant correlations of intramuscular degrees cooled during CWI and adipose thickness and percent body fat respectively. Figures 9 and 10 present slightly less significant correlations of intramuscular degrees cooled post-CWI with adipose thickness and percent body fat respectively. Figures 11 and 12 present highly significant correlations of intramuscular cooling time post-CWI with adipose thickness and percent body fat respectively. Figures 13 and 14 present significant correlations of intramuscular cooling rate post-CWI with thigh volume and tissue depth to bone respectively. Figure 15 presents a highly significant correlation of intramuscular cooling rate post-CWI with thigh circumference.

Table 2. Intramuscular Temperatures During Testing Conditions

	Pre-Bike	Post-Bike	Post-CWI	Lowest Temp	End Temp
Temperature (°C)	35.3 ± 1.2	37.6 ± 0.7	30.8 ± 3.7	29.1 ± 3.0	29.7 ± 2.5

*Values are expressed as means ± SD.

**RM ANOVA significant at $p < .005$

Table 3. Skin Temperatures During Testing Conditions

	Pre-Bike	Post-Bike	Post-CWI	End Temp
Temperature (°C)	30.9 ± 1.1	32.6 ± 1.7	11.2 ± 1.5	22.3 ± 1.5

*Values expressed as means ± SD.

Table 4. Intramuscular Cooling Variables

	CWI	Post-CWI
Cooling Rate (°C/min)	0.27 ± 0.18**	0.13 ± 0.11**
Total Degrees Cooled (°C)	6.9 ± 3.3***	1.7 ± 1.0***
Cooling Time (min)	27.7 ± 4.3	23.0 ± 22.5

*Values expressed as means ± SD.

**Denotes significant difference at $p < .01$.

***Denotes significant difference at $p < .000001$.

Table 5. Intramuscular Cooling Amounts During Testing Conditions

	Degrees Cooled After 10min in CWI	Degrees Cooled After 15min in CWI	Degrees Cooled from Pre-Bike	Degrees Cooled from Post-Bike
Total Degrees (°C)	2.5 ± 2.3	4.0 ± 3.0	6.3 ± 2.0	8.6 ± 2.6

*Value expressed as means ± SD.

** RM ANOVA significant at $p < .0005$

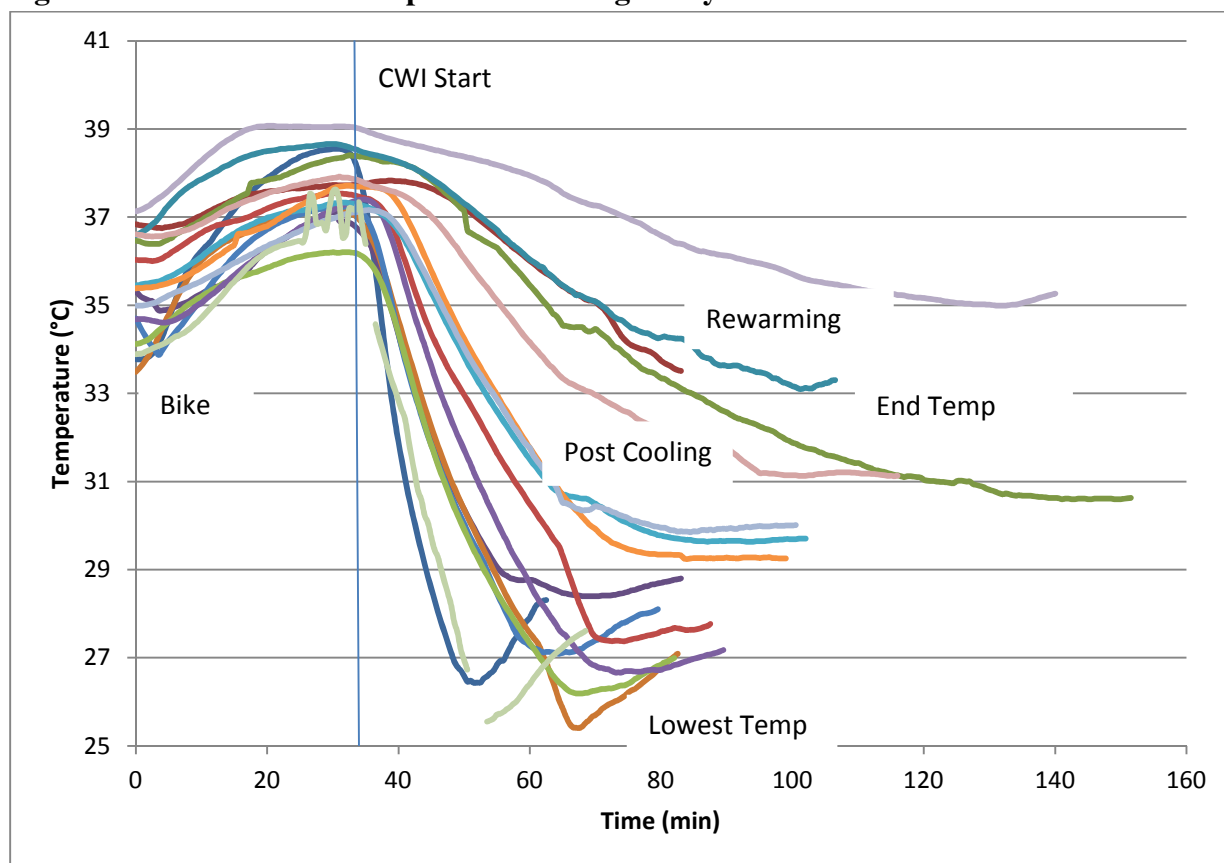
Figure 1. Intramuscular Temperature During Study

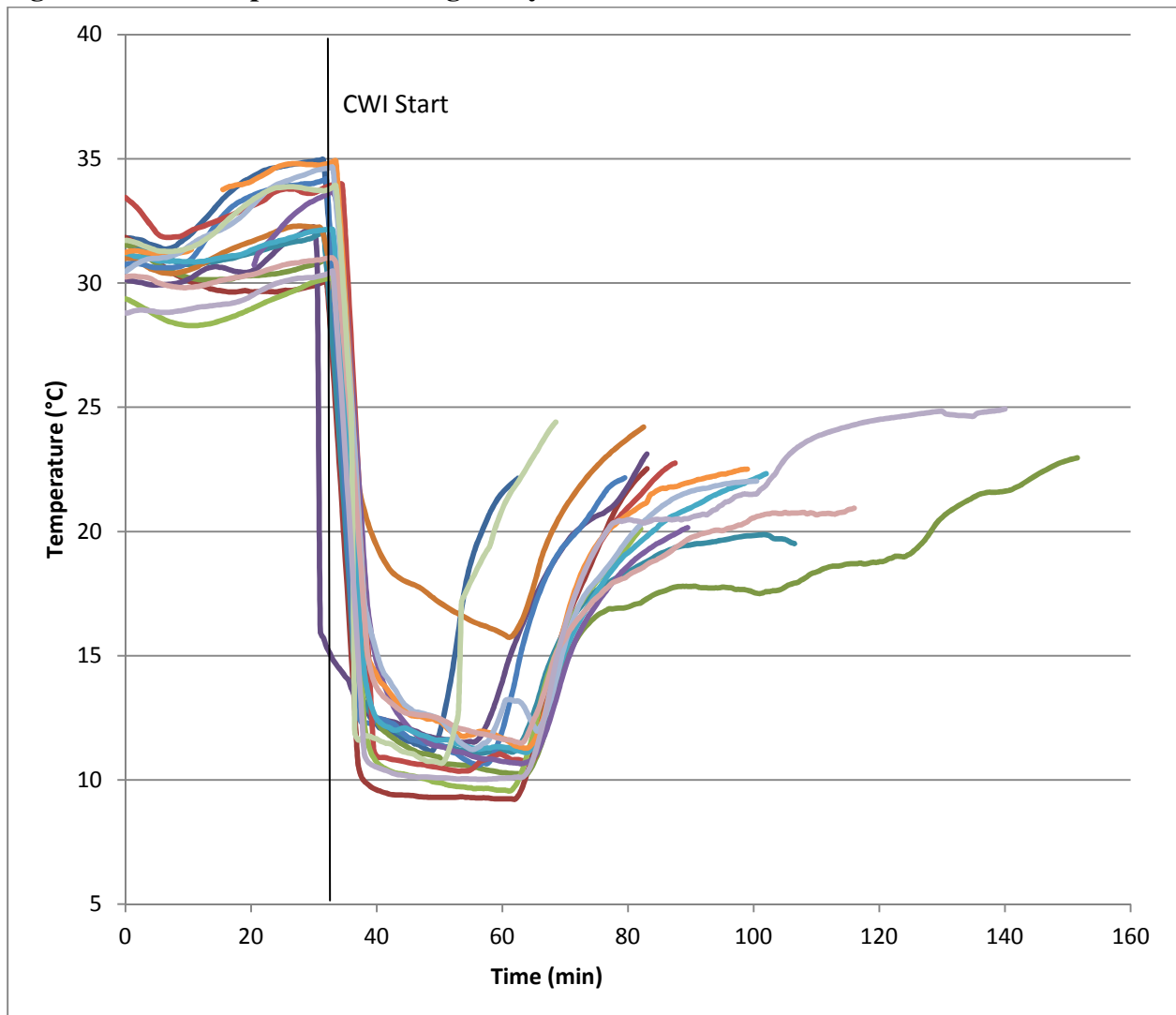
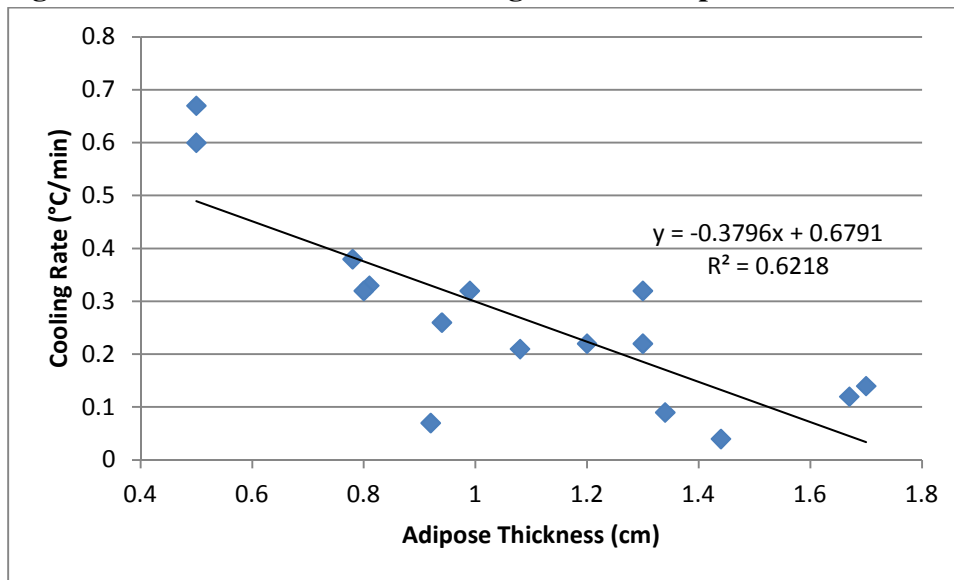
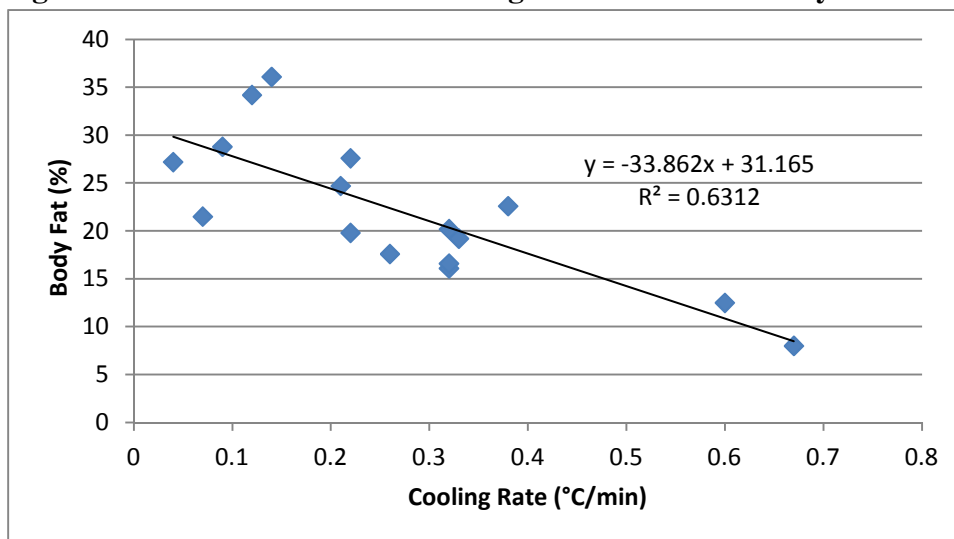
Figure 2. Skin Temperature During Study

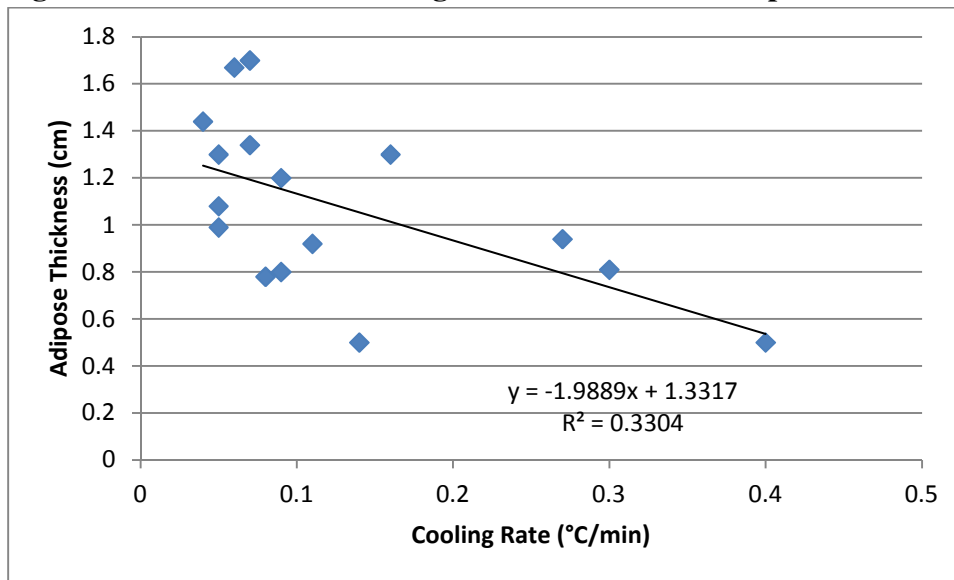
Figure 3. Intramuscular CWI Cooling Rate vs. Adipose Thickness

*Significant at $p < .01$.

Figure 4. Intramuscular CWI Cooling Rate vs. Percent Body Fat

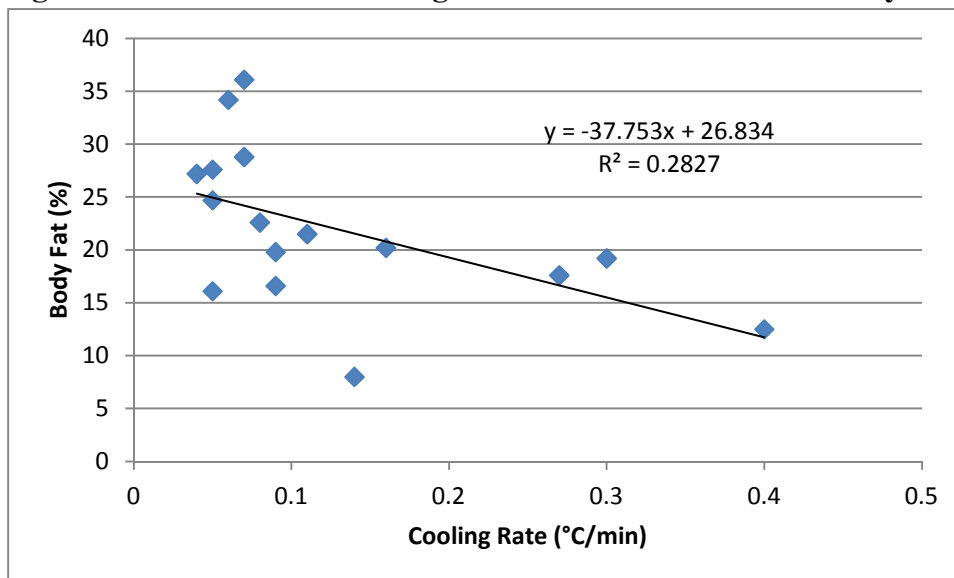
*Significant at $p < .01$

Figure 5. Intramuscular Cooling Rate Post-CWI vs. Adipose Thickness



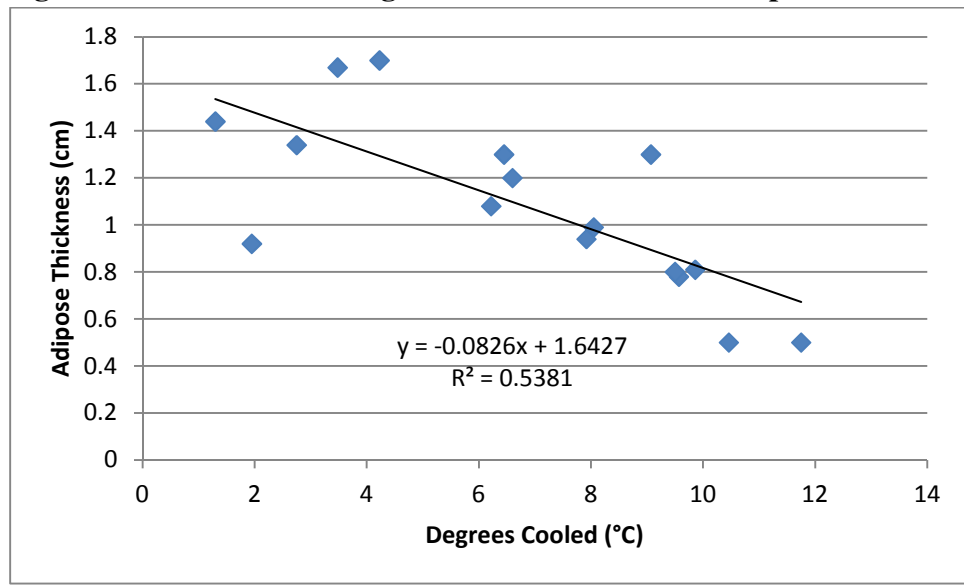
*Significant at $p < .05$. There is less of an effect between post-CWI cooling rate and adipose thickness than was seen between CWI cooling rate and adipose thickness.

Figure 6. Intramuscular Cooling Rate Post-CWI vs. Percent Body Fat



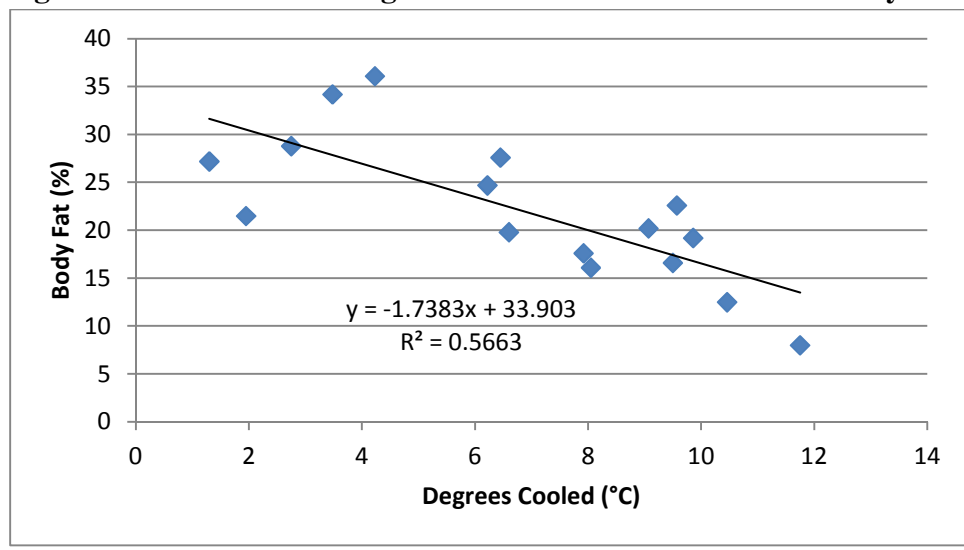
*Significant at $p < .05$. There is less of an effect between post-CWI cooling rate and percent body fat than was indicated between CWI cooling and percent body fat.

Figure 7. Intramuscular Degrees Cooled in CWI vs. Adipose Thickness



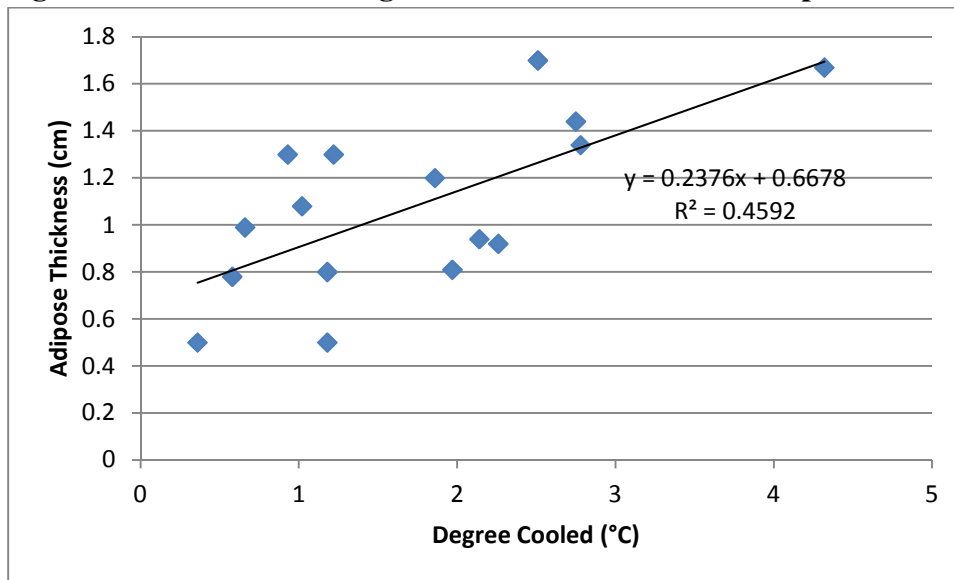
*Significant at $p < .01$.

Figure 8. Intramuscular Degrees Cooled in CWI vs. Percent Body Fat



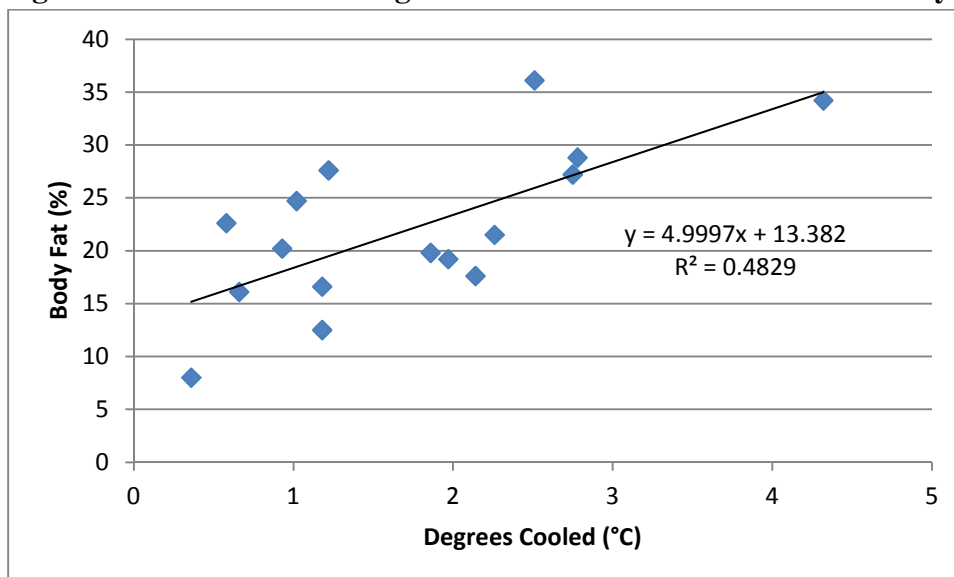
*Significant at $p < .01$.

Figure 9. Intramuscular Degrees Cooled Post-CWI vs. Adipose Thickness

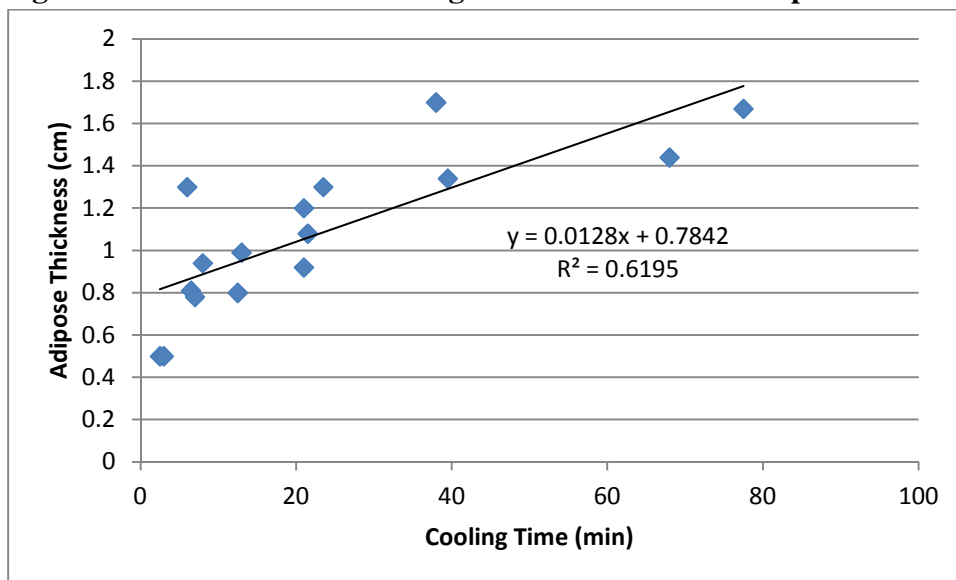


*Significant at $p < .01$. Adipose thickness is slightly less correlated with amount of cooling post-CWI than with amount of cooling during CWI.

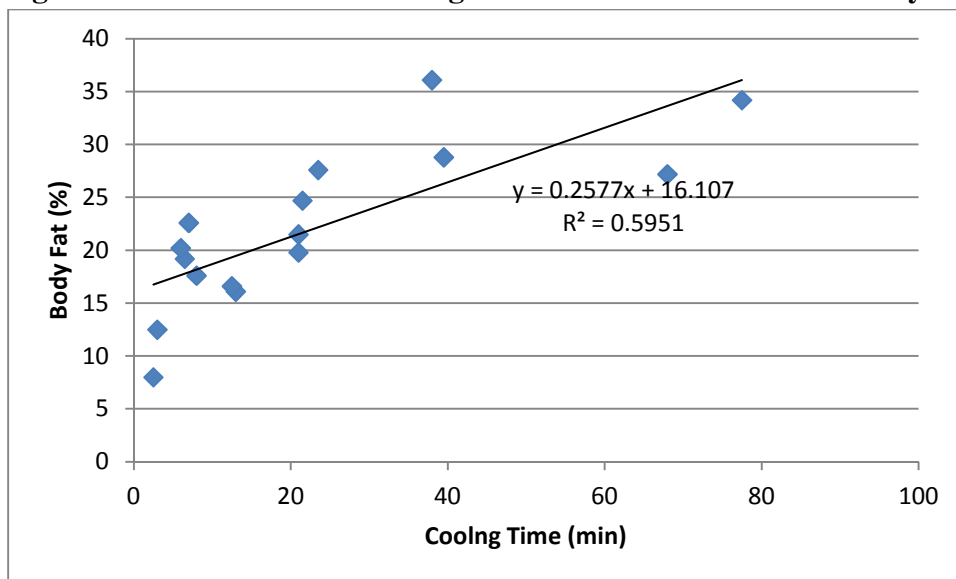
Figure 10. Intramuscular Degrees Cooled Post-CWI vs. Percent Body Fat



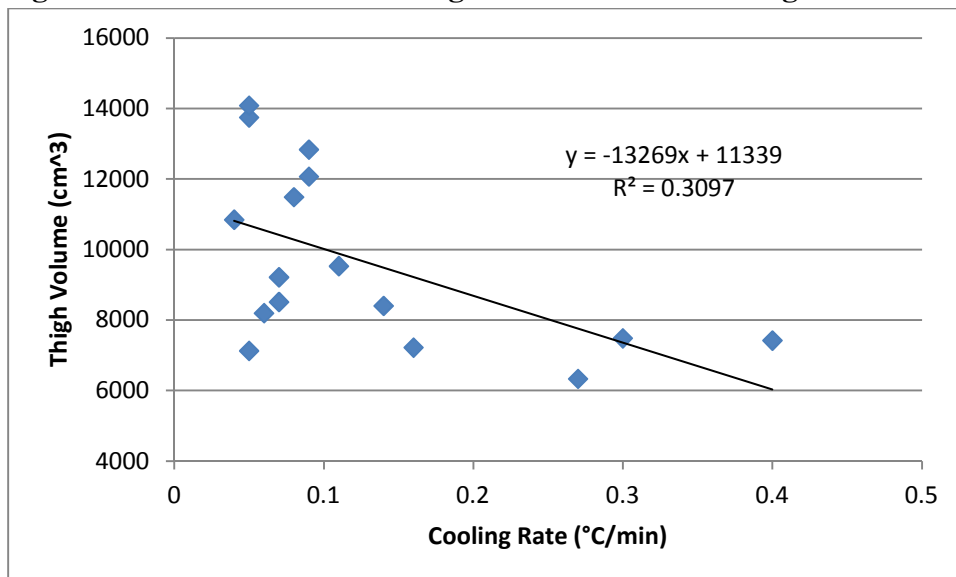
*Significant at $p < .01$. Percent body fat is slightly less correlated with amount of cooling post-CWI than with amount of cooling during CWI.

Figure 11. Intramuscular Cooling Time Post-CWI vs. Adipose Thickness

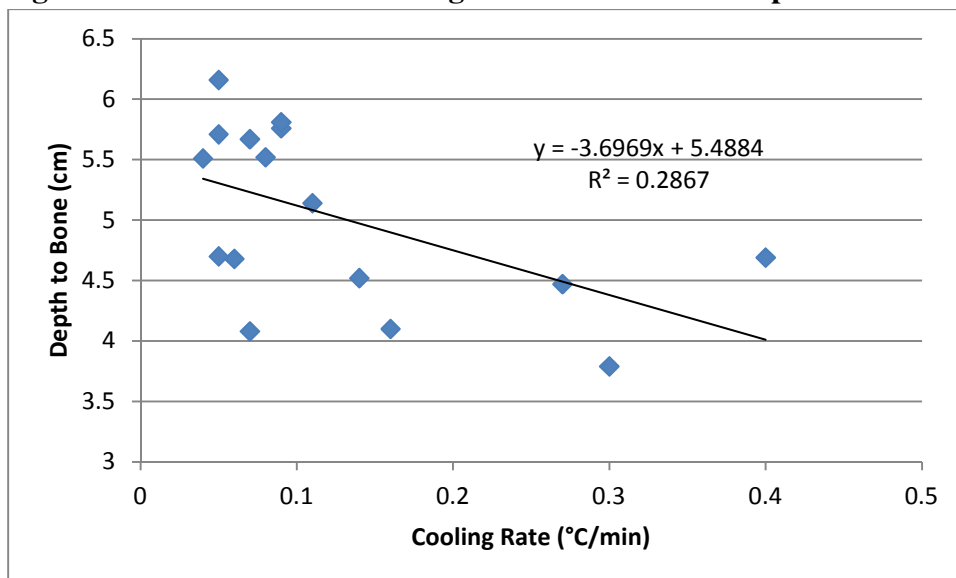
*Significant at $p < .01$.

Figure 12. Intramuscular Cooling Time Post-CWI vs. Percent Body Fat

*Significant at $p < .01$.

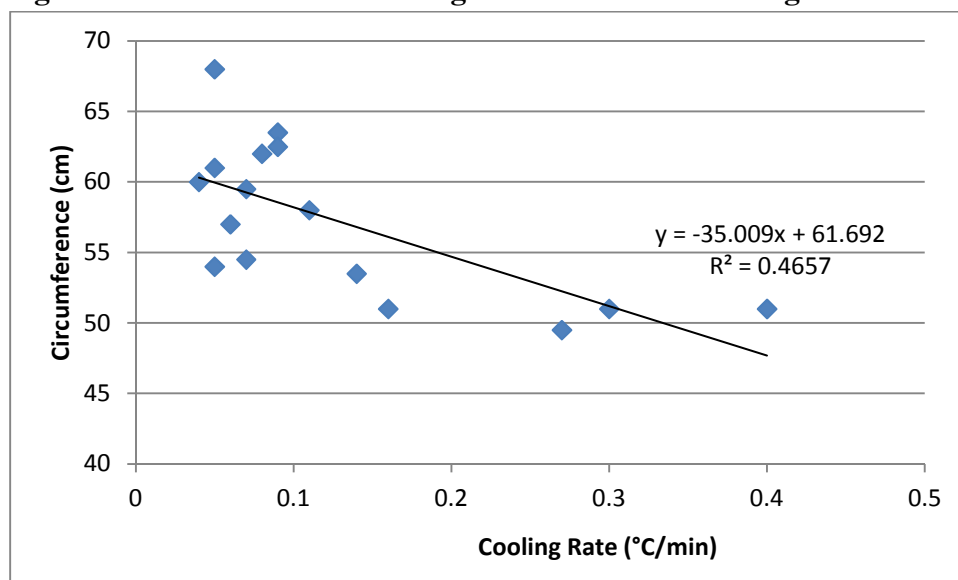
Figure 13. Intramuscular Cooling Rate Post-CWI vs. Thigh Volume

*Significant at $p < .05$.

Figure 14. Intramuscular Cooling Rate Post-CWI vs. Depth to Bone

*Significant at $p < .05$.

Figure 15. Intramuscular Cooling Rate Post-CWI vs. Thigh Circumference



*Significant at $p < .01$.

DISCUSSION

The results agree with previous research indicating a significant inverse relationship between adipose thickness and intramuscular cooling (Myrer et al., 2001; Stocks et al., 2004). Our results have the same trend as Otte et al. (2002) who reported that skinfold thickness played a large role in the ability of an ice bag to drop the intramuscular temperature 1cm sub-adipose tissue by the 7°C recommended by Meeusen and Lievens (1986). In that study a skinfold under 10mm takes 9min to cool, 11-20mm takes 23min, 21-30mm takes 40min and 31-40mm takes 60min to cool muscle. As thigh adipose tissue thickness increase the ability for intramuscular thigh tissue to cool to the recommended 7°C below baseline within a normal treatment time diminishes.

Depth of target tissue is also an important factor that influences cooling. Deeper intramuscular tissue takes longer to cool than more superficial intramuscular tissue. A group of participants (skinfold rang = 10-18mm) received an ice bag treatment to the calf for 20min.

Mean temperature at 1 cm sub-adipose decreased $9.06 \pm 4.0^{\circ}\text{C}$ while mean temperature at 3 cm sub-adipose decreased $3.86 \pm 1.75^{\circ}\text{C}$ (Myrer et al., 2001). Our results indicated a mean temperature reduction at 2 cm sub-adipose tissue of $6.8 \pm 3.3^{\circ}\text{C}$ for up to a 30 min treatment. Although the measurements were taken at two different body sites and with different cold modalities, a trend of reduced cooling as tissue depth increases is present.

A variable not studied by other researchers was percent body fat of the participants. The results of this study show that percent body fat was similarly correlated with cooling as thigh adipose depth. Percent body fat explained 63% of the variance of intramuscular CWI cooling rate compared to 62% explained by adipose depth. Percent body fat was able to explain 56% and 59% of the variability of intramuscular degrees cooled in CWI and intramuscular cooling time post-CWI respectively. Since a thigh skinfold is needed to calculate percent body fat and adipose thickness was just as highly correlated, knowing percent body fat of the participants may not provide any extra value in determining an appropriate CWI protocol time. It does reinforce that the amount of adipose overlying a tissue has a significant negative effect on cooling. CWI protocols based on either adipose depth or percent body fat will accurately cool target tissues to the recommended temperature.

Cooling of tissue at and around the site of acute injury is needed for cryotherapy treatments to be effective. Our study showed that when the thigh is cooled to 2 cm sub-adipose tissue the mean percentage of muscle cooled is estimated to be $35.5 \pm 4.3\%$. Also, only 31% of the participants reached cooling within 30 minutes. In another study approximately 75% of the participants did not cool 8°C below baseline within 30 min using either CWI or a crushed ice bag (Rupp et al., 2012). This indicates that cooling the majority of thigh musculature to the recommended 7°C below baseline is increasingly difficult as adipose thickness increases. The

potential to induce this larger degree of cooling to a majority of the muscle may be restricted to a lean population or body parts with less adipose tissue (Bleakly & Hopkins, 2010).

Thigh intramuscular temperature continued to decrease after treatment. Interestingly, four participants had a greater intramuscular temperature decrease after the CWI treatment than during CWI. The correlation analyses showed thigh circumference, thigh volume and thigh tissue depth to bone, were significantly inversely correlated with cooling rate post CWI. Surface area to mass ratios can have a significant effect on cooling rate (Stocks et al., 2004). Larger thighs have a greater distance to transfer heat and thus will cool at a slower rate. Also, a larger thigh has more tissue to produce heat from metabolism and perfusion to offset cooling. This can be visualized as tissue in the interior of the thigh giving heat to the superficial tissue thus slowing the rate of cooling. In a large thigh the greater volume of warm deep tissue overwhelms the heat transfer system. This seems to indicate that the size of the thigh along with adipose thickness determine the cooling rate post CWI.

However, both thigh adipose thickness and percent body fat are positively correlated with degrees cooled post CWI and time spent cooling post-CWI. This means that increased adipose thickness is associated with greater temperature reductions post-CWI as well as longer lasting cooling. What we suggest is happening is that the adipose is acting as insulation inhibiting heat transfer to the superficial musculature. An individual with minimal thigh adipose will rewarm quickly because their tissue will be receiving heat from both the ambient temperature of the environment as well as deep tissues. For an individual with thicker adipose, heat transfer to superficial muscle by the ambient environment will be diminished thus allowing that tissue to stay cooler and act as the final heat acceptor. By keeping the final heat acceptor as superficial as possible, cooling can be carried further into the thigh.

As thigh musculature is cooled deeper tissues are cooled by losing heat to more superficial tissues. This can be thought of as heat being passed from deep tissues out to the cold modality which is the final heat acceptor. Adipose tissue acts as an inhibitor to the transfer of heat. It takes a certain buildup of superficial cooling until heat transfer can continue past the adipose tissue layer. This means that individuals with larger amounts of adipose tissue will take longer to cool intramuscularly.

When the cold modality is removed heat transfer at the superficial thigh is reversed and now the final heat acceptor is somewhere in the interior of the thigh. Just as adipose tissue inhibited cooling when cold was applied, it inhibits rewarming when cold is removed. Therefore, once sub-adipose tissue has cooled it will take longer for that tissue to rewarm. It should also be noted that metabolism and perfusion of the muscle are constantly combating the heat transfer of the cold modality. So the heat loss from the cold treatment must overcome the heat gain from metabolism and perfusion. Using this model, cooling depends of depth of target tissue and thickness of the overlying adipose tissue. This is assuming there are no changes in metabolism and perfusion other than caused by the cold modality. Bender et al. (2005) demonstrated that walking with an ice bag wrapped to the calf significantly impairs cooling of the cold modality compared to resting with an ice bag on the calf.

Vasodilation and blood volume redistribution toward the legs results in accelerated heat loss (Stocks et al., 2004). The only other study to look at post-exercise cooling was conducted by Long et al. (2005) where after 30 minutes of riding a stationary cycle ergometer, participants had an ice bag placed on their anterior thigh until the tissue 2 cm sub-adipose was cooled 10°C below baseline temperature. They found that cooling was enhanced after exercise compared to cooling from a resting temperature. Their participants had a similar adipose thickness (skinfold = 25.4 ±

2.7 mm) to the participants in our study and the time to cool at the 2 cm depth was 39.6 ± 7.1 min. This is approximately a rate of $.25$ °C/min which is quite similar to the rate in our study (0.27 ± 0.18 °C/min). Crushed ice as normally been reported to produce a greater magnitude of cooling in a given time than CWI. Long et al. (2005) required a greater amount of cooling which may account for the similarity in CWI cooling rates. The graphs presented by Long et al. (2005), depict participants cooling to 7 °C below baseline in approximately 25 min. Crushed ice at 0 °C has a greater temperature gradient than CWI and therefore has more potential to extract heat from the tissue (Bleakley & Hopkins, 2010). In contrast the majority of the participants in our study did not reach the adequate cooling of 7 °C below pre exercise temperature within 30 min. This would seem to indicate that if cooling is directed at a specific area of the thigh; a larger temperature reduction can be attained using an ice bag. However this may not be sufficient to cool enough musculature to alleviate symptoms of exercise induced muscle soreness.

In another study by Merrick et al. (2003) participants (skinfold = 19.3 ± 4.1 mm) received an ice bag, wet-ice, or flex-i-cold treatments for 30 min to their anterior thigh with no prior exercise. The wet-ice treatment which was a commercially available ice pack showed the greatest temperature reduction of about 5.5 °C over the 30 min treatment. This is less than the 6.3 ± 2.0 °C mean temperature reduction from baseline during CWI observed in our study. Long et al., (2005) noted that prior exercise results in a faster cooling rate than cooling from resting temperature. This was attributed to an increase in the temperature gradient between the cooling modality and the skin. The participants in our study exercised before cooling which may explain why greater cooling was seen.

Similar to our study, Myrer et al. (1998) compared a cold whirlpool (10 °C) with an ice pack treatment to the calf muscles for 20min. A greater temperature reduction was seen in the ice

pack treatment but post treatment the intramuscular temperature of the ice pack treatment began to rewarm while the cold whirlpool treatment continued to cool for approximately 25 min with a total post-treatment reduction of $1.8 \pm 1.4^{\circ}\text{C}$. Our study demonstrated a significant post-treatment reduction of intramuscular temperature of $1.7 \pm 1.0^{\circ}\text{C}$ over 23 ± 22.5 min. This may indicate that while ice packs may produce a lower intramuscular temperature during treatment, the sustained temperature reductions brought about by CWI may be more effective at providing adequate cooling.

Rupp, Herman, et al. (2012) looked at cooling of the calf muscle to 8°C below resting temperature using CWI and a crushed ice bag. The CWI treatment took nearly 40 min to cool intramuscular temperature at 1 cm sub-adipose by 8°C . Since our study was looking at 2 cm sub-adipose and only five of the participants were able to cool within 30 min this is in line with previous studies indicating deeper tissues take longer to cool than more superficial ones (Merrick et al., 2003; Myrer et al., 2001). The CWI treatment was also significantly colder at 90min post-treatment than the crushed ice bag group. This is in line with our findings that there is significant intramuscular cooling post-treatment with CWI. They did not see an interaction between cooling and adipose thickness which goes against our results. This may have been due to a lack of variability in there skinfolds.

Multiples studies (Bailey et al., 2007; Ingram et al., 2008; Jakeman et al., 2009; King and Duffield, 2009; Rowsell et al., 2009) used CWI protocols of 10°C , as was used in our study. Since treatment was also done after exercise a similar cooling rate to our study is reasonable to expect. However, the longest duration was only 15 min and two of the studies used protocols that did not have the participants continuously in CWI but alternated between immersion and time out of water (King & Duffield, 2009; Rowsell et al., 2009). As discussed previously heat transfer

is reversed when the cold modality is taken away. Even though it has been demonstrated that cooling continues after CWI treatment, the intermittent reversal of heat transfer when participants leave CWI undermines the cooling effects of CWI. Jakeman et al. (2009) and King and Duffield, (2009) showed no improvement of indices of EIMD.

Bailey et al. (2007) produced some of the best improvements in markers of EIMD (decreased myoglobin, decreased soreness and increased contraction force of knee flexors). However, there was not a significant improvement of the knee extensor contraction force or CK levels. A longer treatment duration may have improved the results our results indicate that adequate cooling of the muscle probably did not take place within the 10 min treatment.

Our results indicate that the average temperature reduction after 10 and 15 min in CWI was $2.5 \pm 2.3^{\circ}\text{C}$ and $4.0 \pm 3.0^{\circ}\text{C}$ respectively. It is clear that studies to date are not using protocols that are eliciting a temperature reduction that is near what is needed to reduce tissue metabolism by 2 to 3 fold. Minimal decreases in intramuscular temperature are achieved after 10-15 min of CWI, which is the length of CWI normally used in the clinical setting. CWI after an acute injury, using previous clinical protocols, will likely not yield the large temperature reductions necessary to prevent secondary hypoxic injury. Yet studies using these durations see positive results in reduced CK, muscle soreness, edema and other markers of EIMD.

Therefore it may be that the temperature reduction needed after acute injury to prevent secondary hypoxia is different than what is needed to alleviate some of the symptoms of EIMD after a workout. A distinction between the goals of CWI needs to be made prior to treatment. CWI used after a strenuous work out as a recovery modality may provide benefits when immersion duration is 10-15 min. However, if a more serious acute injury is sustained beyond

what could be considered normal EIMD, a CWI duration of approximately 30 min on average is needed to decrease tissue temperature to the recommended amount. Again cooling is going to be based on the adipose thickness and depth of target tissue for each individual.

CONCLUSION

It may not be possible to cool enough of the thigh musculature to an adequate temperature that reduces tissue metabolism by 2-3 times for most individuals in a treatment period shorter than 30 min. In most individuals with thigh adipose tissue greater than 1 cm tissue intramuscular cooling will not be enough to reduce metabolism by the recommended amount. Cold modality protocols should be based on adipose thickness and depth of target tissue because these have been shown to significantly influence cooling. However, this degree of tissue cooling may not be needed to alleviate at least some of the markers of muscle damage after a workout as seen by the positive results of other studies. Future research into the optimal post-workout cooling amount for reduction of EIMD is needed. A study comparing a normal CWI duration of 10-15 min with a duration based on time to cool 7°C below baseline temperature on indices of EIMD would enhance our understanding of how to cool muscle for post-workout recovery purposes. Research may also be directed to look at differences in cooling with participants accustomed to CWI and those who are unaccustomed. It would be good to know if a physiologic response develops in people who regularly receive CWI treatment. If tolerance to cooling can be built up, using CWI after every practice or workout may be contraindicated because it would reduce the cooling effects at times when greater cooling is imperative.

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