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Local muscle cooling does not impact expression of mitochondrial-related genes

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ABSTRACT

Recovery that takes place in a cold environment after endurance exercise elevates PGC-1α mRNA whereas ERRα and NRF2 mRNA expression are inhibited. However, the effect of local skeletal muscle cooling on mitochondrial-related gene expression is unknown.

Purpose: To determine the impact of local skeletal muscle cooling during recovery from an acute bout of exercise on mitochondrial-related gene expression.

Methods: Recreationally-trained male cyclists (n = 8, age 25 ± 3 y, height 181 ± 6 cm, weight 79 ± 8 kg, 12.8 ± 3.6% body fat, VO_{2peak} 4.52 ± 0.88 L·min^{-1} protocol) completed a 90-min variable intensity cycling protocol followed by 4 h of recovery. During recovery, ice was applied intermittently to one leg (ICE) while the other leg served as a control (CON). Intramuscular temperature was recorded continuously. Muscle biopsies were taken from each *vastus lateralis* at 4 h post-exercise for the analysis of mitochondrial-related gene expression.

Results: Intramuscular temperature was colder in ICE (26.7 ± 1.1 °C) than CON (35.5 ± 0.1 °C) throughout the 4 h recovery period ($p < 0.001$). There were no differences in expression of PGC-1α, TFAM, NRF1, NRF2, or ERRα mRNA between ICE and CON after the 4 h recovery period.

Conclusion: Local muscle cooling after exercise does not impact the expression of mitochondrial biogenesis-related genes compared to recovery from exercise in control conditions. When these data are considered with previous research, the stimuli for cold-induced gene expression alterations may be related to factors other than local muscle temperature. Additionally, different intramuscular temperatures should be examined to determine dose-response of mitochondrial-related gene expression.

1. Introduction

Mitochondrial dysfunction has been implicated in peripheral arterial disease (Makris et al., 2007), aging (Derbré et al., 2012), obesity (Bournat and Brown, 2010), and other diseases (Bullon et al., 2014). Therefore, treatment options to increase mitochondrial function are of great interest. Exercise is effective at stimulating mitochondrial biogenesis (Wright et al., 2007; Baar et al., 2002). Additionally, exercise and subsequent recovery from exercise in a cold environment alters the gene expression of transcription factors associated with mitochondrial development (Slivka et al., 2013). One of the key regulators in mitochondrial biogenesis is peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). PGC-1α expression is stimulated in skeletal muscle with exercise (Baar et al., 2002; Pilegaard et al., 2003) and with cold exposure (Liang and Ward, 2006; Wu et al., 1999). Expression of PGC-1α is further enhanced with exercise during cold exposure in rats and humans (Seebacher and Glanville, 2010; Slivka et al., 2012, 2013). However, the mRNA of transcription factors that are co-activated by PGC-1α decrease or are unaffected after exercise and subsequent recovery in a cold environment (Slivka et al., 2013). The apparent paradoxical response of PGC-1α and other transcription factors related to mitochondrial development after exercise in a cold environment indicate a cold-induced alteration in the typical exercise response. Furthermore, transcription factors downstream of PGC-1α such as mitochondrial transcription factor A (TFAM), nuclear respiratory factor-1 (NRF1), nuclear respiratory factor-2 (NRF2), and estrogen-related receptor alpha (ERRα) are also important in mitochondrial development.

Cold exposure immediately following exercise is a commonly used method to facilitate recovery. Utilizing cold-based recovery techniques after an acute bout of endurance exercise is commonly used in hopes of quicker recovery and enhanced performance in subsequent exercise

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sessions (White and Wells, 2013). Ice therapy has been used in the treatment of acute injuries and is often included in the recovery process for exercise-induced muscle damage with the intention of enhancing recovery (Oakley et al., 2013). Ice therapy may also be an easily accessible way to prevent or manage mitochondrial dysfunction in a clinical population. However, the efficacy of cryotherapy has had mixed results as a recovery modality. Local cooling attenuates anabolic hormones (Nemet et al., 2009), decreases neuromuscular function (Peiffer et al., 2009), and adversely affects functional performance tests immediately after icing (Fischer et al., 2009). Potential benefits of local cooling include providing analgesic effects (Oakley et al., 2013), reducing inflammatory cytokines (Nemet et al., 2009; Oakley et al., 2013), and delaying fatigue when applied pre-exercise or during exercise (Kwon et al., 2013). The effects of cryotherapy on glycogen re-synthesis after exercise has produced controversial results (Tucker et al., 2012; Slivka et al., 2013). Specifically, local muscle cooling reduces glycogen re-synthesis yet environmental cold exposure does not affect glycogen re-synthesis. It is unclear if these cold-induced effects are stimulated similarly during other types of cold stress, because exposure to environmental cold may elicit different physiological responses than the application of local cold such as icing. Environmental cold exposure causes systemic effects (decreased core body temperature) whereas local cold application over a relatively smaller area, may only produce local alterations (Tucker et al., 2012).

We have previously reported the effects of exercise and subsequent recovery in a cold environment on gene expression related to mitochondrial development. However, limited knowledge exists on the effects of local muscle cooling on the exercise-induced transcriptional response related to mitochondrial development. Thus, the purpose of this study is to determine the impact of local muscle cooling after exercise on gene expression related to mitochondrial development. These data may determine the effectiveness of using local cooling during recovery after endurance exercise. Furthermore, these treatment options may serve as a potential therapy and ameliorate the deleterious effects of mitochondrial dysfunction.

2. Methods

2.1. Ethics statement

Participants completed a Physical Activity Readiness Questionnaire (PAR-Q) and were briefed on the experimental protocol and possible risks prior to giving informed consent. All procedures were approved by the Institutional Review Board.

2.2. Body composition

Body density was measured using hydrodensitometry corrected for estimated residual lung volume and gastrointestinal air volume (Quanjer et al., 1993), and then converted to percent body fat using the Siri equation (Siri, 1993). Participants were instructed to completely submerge themselves in the hydrostatic weighing tank, expel the air in their lungs, and remain still for three seconds while their underwater weight was recorded using a calibrated electronic load cell based system (Exertech, Dresbach, MN). Six to ten trials were performed and the three best trials were averaged to estimate percent body fat.

2.3. VO_{2peak}

A graded exercise test was completed to measure maximal aerobic capacity. This test was performed on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA) using a gas and flow calibrated metabolic cart (ParvoMedics TrueOne Metabolic System, Sandy, Utah) to measure expired gases. The graded cycle test started at 95 W and increased by 35 W after every three minutes. The participants cycled until volitional fatigue and the highest VO_2 obtained during the test was recorded as VO_{2peak}. Maximum workload associated with VO_{2peak} (W_{max}) was determined by taking the highest completed stage (in watts) and adding the proportion of time completed in the last stage multiplied by the 35 W per stage increment. The graded cycle protocol was used to establish intensity for the experimental trial.

2.4. Experimental trial

Participants arrived after a 12 h fast and having refrained from exercise within the previous 24 h. A single trial was performed consisting of an exercise bout and a four-hour recovery period. The exercise bout consisted of a 10 min warm up at ~55% VO_{2peak} followed by 10 intervals of two min at ~80% VO_{2peak} and four min at ~50% VO_{2peak}. After the 10 intervals, subjects completed 12 min at ~60% VO_{2peak} and 10 min at ~50% VO_{2peak}. These intensities were based on the percentage of W_{max} that corresponded with VO_2 during the graded test. Subjects were allowed to drink 590 mL of water during the 90 min cycling bout. Immediately after cycling, subjects toweled off and changed into dry clothing. Participants then rested in the supine position for four hours with one leg randomly assigned as the experimental iced leg (ICE) and the other as the control leg (CON).

2.5. Icing protocol

Two 1 kg, thin plastic bags of crushed ice were placed along the *vastus lateralis* of the iced leg. Small pillows were placed next to the leg to help keep the bags in place without providing compression. Ice remained on the leg for the first hour of recovery and then was removed and reapplied at 30 min intervals for the remainder of the 4 h recovery period. Four hours was chosen as this has been an appropriate period for the peak gene expression of the selected mitochondrial-related genes assessed in this study (Pilegaard et al., 2003; Leick et al., 2010).

2.6. Core temperature

Core temperature was monitored continuously during the recovery period with a rectal thermistor that was inserted 15 cm past the anal sphincter and recorded with a digital data logger (Physitemp Instruments Inc., Clifton, NJ).

2.7. Intramuscular temperature

To measure intramuscular temperature, an 18-gauge introducer needle was inserted into the *vastus lateralis*. The needle was then retracted, leaving a flexible removable Teflon catheter (Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT). A thermocouple (1 mm diameter; Physitemp Instruments Inc.) was fed into the indwelling catheter to 4.3 cm past the skin. Once the thermocouple was in place the introducer catheter was removed leaving the thermocouple in place. The thermocouple was then secured to the skin and covered with a 6 × 7 cm adhesive dressing (Tegaderm, 3 M, St. Paul, MN). Recordings were collected every second and averaged every 15 min during recovery with an electronic temperature data logging system (Physitemp Instruments Inc.). Time point 0 min represents the initial intramuscular temperature before the intervention of the cold packs. Time point 0 min occurred approximately 15–20 min after the exercise protocol was complete (the time required to place temperature probes). The recovery portion of the trial began with intramuscular probes placed and subjects resting in the supine position.

2.8. Muscle biopsies

Muscle biopsies were taken from the *vastus lateralis* of both legs 4 h after exercise using the percutaneous biopsy needle technique with the aid of suction (Bergström, 1962). Biopsies on the iced limb and the