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ORIGINAL ARTICLE

Cold-water immersion blunts and delays increases in circulating testosterone and cytokines post-resistance exercise

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Abstract

Introduction Cold-water immersion (CWI) is often used to promote recovery by reducing exercise-induced muscle damage, soreness, and inflammation. However, recent reports have cautioned that CWI may attenuate the adaptive response to resistance training.

Purpose To determine the effect of post resistance-exercise CWI on circulating free testosterone (T) and cytokine (IL-6 and TNF-α) response.

Methods Using a randomized and counterbalanced repeated-measures design, 11 resistance-trained men completed two workouts (6 sets of 10 repetitions of back squats at 80% of maximum load) a week apart after which they took part in either 15 min of CWI (15 °C) or passive recovery. T, IL-6, and TNFα were measured in blood samples taken before (PRE) and 5 (5POST), 15 (15POST), 30 (30POST), and 60 (60POST) min post-exercise and compared between treatments and over time.

Results For T, a significant interaction effect of condition over time ($p = 0.030$) as well as greater relative concentrations of T in CON (Δ9.2%) than CWI (Δ-0.5%, $p = 0.049$) at 30POST were observed. In addition, at 60POST, T dropped below PRE values in CWI (Δ-10.4%, $p = 0.028$) but not in CON (Δ-1.6%, $p = 0.850$). A suppressed cytokine response was observed after CWI in IL-6 at 30POST (CWI: Δ4.9%, CON: Δ47.5%, $p = 0.041$) and TNFα at 15POST (CWI: Δ5.3%, CON: Δ17.0%, $p = 0.022$).

Conclusions CWI blunted the T and cytokine response after a bout of resistance exercise. These results indicate that CWI results in an altered anabolic response and may help to explain the previous observation of attenuated hypertrophy when CWI is used after resistance exercise.

Keywords Cryotherapy · Inflammation · Hypertrophy · Anabolic · Recovery · Ice

Abbreviations

1RM	Back squat one repetition maximum
BM	Body mass
CON	Passive control condition
CWI	Cold-water immersion condition
IL-6	Interleukin-6
POST	Post-exercise time point (5, 15, 30 or 60 min)
PRE	Pre-exercise time point
T	Free testosterone
TNFα	Tumor necrotic growth-factor alpha

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Introduction

Cryotherapy is a commonly used post-exercise recovery modality that often takes the form of cold-water immersion (CWI: Wilcock et al. 2006). Cold-water immersion has been shown to reduce markers of exercise-induced muscle damage (Eston and Peters 1999), delayed onset muscle soreness (Abaidia et al. 2017; Versey et al. 2013), and inflammation (Crystal et al. 2013; Ziemann et al. 2012) while increasing the rate at which strength and power are recovered after exercise (Abaidia et al. 2017; Roberts et al. 2017; Versey et al. 2013). To the contrary, these results suggest that chronic use of CWI should theoretically increase an individual's training

capacity and ultimately lead to greater training adaptations. To the contrary, several studies have observed attenuated strength (Burke et al. 2010; Frohlich et al. 2014; Yamane et al. 2006) and hypertrophy (Roberts et al. 2015) adaptations when CWI is performed after resistance exercise.

Roberts et al. (2015) observed that when CWI is performed after resistance exercise, compared to active recovery, it results in an acute suppression of the mTOR pathway via a reduction of p70S7 kinase phosphorylation. This suppression was also related to diminished hypertrophy and satellite cell proliferation after an 8-week resistance-training intervention. While these results suggest that acute suppression of the mTOR pathway can negatively affect chronic resistance-training adaptations, less is known about the effects of CWI on upstream regulators of these pathways such as testosterone (T) and the cytokines interleukin-6 (IL-6) and tumor necrotic growth factor alpha (TNFα).

Testosterone is well established as a potent anabolic hormone as demonstrated by the effectiveness of pharmacologically administered T, clinical effects in hypogonadal populations, and a stimulatory effect on anabolic pathways such as mTOR (Basualto-Alarcon et al. 2013; Hooper et al. 2017; White et al. 2013). Because studies investigating the effects of chronic resistance training on basal T have shown mixed results, there has recently been increased interest in the acute post-resistance exercise T response prior to down-regulation of cellular receptors (Hooper et al. 2017; Urhausen et al. 1995). The previous studies have found that resistance-exercise results in a pulsatile release of T from the testees that generally return to pre-exercise values within an hour of completion of the exercise. In males, the temperature of the testes is independently regulated from core temperature via superficial blood vessels and the temperature-sensitive scrotal muscle dartos, which constricts upon exposure to cold and has been shown to be sensitive to chronic cold stress (Grasso et al. 2014; Juarez-Rojas et al. 2015). Such constriction could reduce or occlude blood flow to the testes during or acutely after CWI and, thus, reduce bioavailability of T and ultimately the body's natural anabolic response (Kraemer et al. 2017).

The mTOR pathway has many upstream regulatory mechanisms other than T that may be affected by CWI (Morita et al. 2015). Of particular interest are the cytokines IL-6 and TNFα, which have been found to be related to both mTOR regulation and inflammatory response (Crystal et al. 2013; Febbraio and Pedersen 2002; Ziemann et al. 2012). Presently, several studies have observed the role of CWI on post-resistance exercise cytokine response (Peake et al. 2017; Roberts et al. 2014; Ziemann et al. 2012). From these studies, it has been observed that CWI blunts the IL-6 and TNFα response 2 h and 2 days after resistance exercise when compared to general active recovery (Peake et al. 2017; Roberts et al. 2014; Ziemann et al. 2012). Presently, only one study has investigated the post-resistance-exercise IL-6 response in time periods shorter than 2 h (Roberts et al. 2014). This study found IL-6 increased at 15, 60, 90, and 120 min post-resistance after both CWI and active recovery, but IL-6 concentrations 90 and 120 min post-exercise were significantly lower after CWI than active recovery. No studies have investigated the TNFα response in time periods less than 2 h post-resistance exercise.

The purpose of the present study is to determine if CWI affects circulating T, IL-6, and TNFα concentrations within 1 h of resistance exercise (Morita et al. 2015; Wang and Proud 2006; White et al. 2013). Based on the rationale above, we hypothesize that post-resistance training CWI will result in acute suppression of the T, IL-6, and TNFα responses when compared to passive recovery.

Methods

Subjects

Eleven men participated in this study [age: 21.1 ± 2.1 year, height: 1.76 ± 0.09 m, body mass (BM): 76.2 ± 10.5 kg]. All participants were considered resistance trained, which was defined as having continually resistance trained at least twice per week over the last year and being able to perform the parallel-depth (thigh parallel to the ground) back squat lift with at least 1.5 times BM [one repetition maximum (1RM): 126.0 ± 18.9 kg: 1RM:BM: 1.65 ± 0.14]. All participants habitually performed back squats as part of their resistance training routine and were free of any musculoskeletal injuries that would prevent them from performing heavy back squats or hormonal disorders. This study was approved by the institutional ethics committee at the University of Rhode Island and all participants gave their informed written consent prior to participation and after having the risks and benefits of the study carefully explained to them.

Experimental design

This study utilized a randomized crossover design, where each participant performed a strength testing session as well as two experimental sessions in which one of two post-exercise interventions (CWI or no intervention) were randomly assigned using a random number generator with condition order being counterbalanced between subjects. In the strength testing session, participants performed a 1RM test per for the parallel-depth Smith machine back squat exercise to confirm their eligibility for the study as well as to determine their exercise loads for the two experimental sessions (Kraemer et al. 2005). During the two experimental sessions, participants performed a fatiguing resistance-exercise intervention after which they received one of two recovery

interventions: (1) 15 min of cryotherapy in the form of CWI or (2) 15 min of passive recovery with a matched body position. Prior to and 5, 15, 30, and 60 min post-resistance exercise T, IL-6, TNFα, and lactate (LA) were measured from venous blood. Comparisons were then made between testing conditions and between time-points.

All experimental sessions were performed between 6:30 and 9:00 a.m. For each participant, the two experimental sessions were separated by at least 3 but no more than 14 days. Prior to each testing session, participants were instructed to and confirmed that they had avoided any exercise or strenuous physical activity for at least 48 h, avoided alcohol, caffeine, and any other depressant or stimulant for at least 12 h, and fasted 8 h prior to testing. All participants were instructed to attend each experimental session euhydrated which was confirmed by urine specific gravity ≤ 1.020.

Strength testing

All participants completed a strength testing session to confirm that they possessed the prerequisite exercise technique and strength for the study as determined by a 1RM test for the Smith machine back squat (Kraemer et al. 2005). Prior to testing, each participant performed a standardized warm-up consisting of 5 min of cycling on a bike ergometer (Monark 828E, Monark Exercise AB, Vansbro Sweden) at a low intensity (1.5 kP at 60 rpm) as well as a series of dynamic stretches which consisted of ten repetitions each of body weight squats, lunges, knee hugs, quadriceps pulls, and leg swings. Afterwards, participants performed an exercise specific warm-up in which they performed four submaximal sets of parallel-depth Smith machine back squats with increasing loads (ten repetitions with an empty bar, ten repetitions with 50% of estimated 1RM load, five repetitions with 75% estimated 1RM load, and one repetition with 90% of estimated 1RM load).

After the standardized warm-up, participants were given up to six attempts to determine their 1RM. A successful attempt was recorded when the participant was able to actively lower and raise the bar from parallel depth (top of thigh's parallel to the ground) without assistance and in a safe and controlled manner. Each attempt was separated by 3 min of passive recovery and the largest load successfully lifted was defined as their 1RM.

Resistance-exercise protocol

For each of the two experimental sessions, after pre-exercise (baseline) measurements were obtained, participants replicated the standardized warm-up previously described. Participants were then given 5 min of passive recovery prior to the start of a high intensity resistance-exercise workout. The resistance-exercise protocol consisted of performing six sets of ten repetitions of parallel-depth Smith machine squats with 80% of their previously determined 1RM with an interest rest period of 2 min (Roberts et al. 2014). During the first experimental session, exercise load was reduced between sets when necessary to ensure ten repetitions were performed using proper form during the subsequent set and the 2 min rest period was maintained. In cases where load was reduced or not all repetitions were performed during a given set, during the second experimental session, identical loads and repetitions were performed to match exercise load (i.e., total work done). To determine if exertion was different between conditions, a rate of perceived exertion for resistance exercise (CR-10) was collected immediately after the final set of exercise and compared between exercise bouts.

Recovery intervention

Immediately after the last set of the resistance-exercise protocol, participants sat in a wheel chair and were transported to a recovery room where a 5 min post-exercise blood draw was obtained prior to the participant receiving one of two post-exercise interventions: (1) CWI or (2) passive recovery (CON). During the CON intervention, participants sat on an inclined (120°) training table for 60 min, with their legs slightly flexed to prevent vascular occlusion and match the position which they would receive the CWI intervention. Post-exercise measurements were then taken in this position at 15, 30, and 60 min post-exercise. During the CWI intervention, participants removed all clothing except for their compression shorts prior to entering a 100-gallon stock tub (Rubbermaid, Winchester, VA, USA) which was prefilled with 15 °C water up to the level of the participant's xiphoid process. In the tub, participants were positioned, so that they could relax their body and keep their legs passively flexed and fully submerged. During the intervention, water temperature was constantly monitored and maintained at 15 °C. Participants were successfully discouraged from shivering and instructed to remain as relaxed as possible during the intervention. The CWI temperature and duration used in the present study selected from the range, which has shown to have the greatest effect on muscle soreness (11–15 °C for 11–15 min: Machado et al. 2016). After 15 min of treatment, participants were assisted out of the tub, towel dried, and allowed to quickly change clothes prior to sitting on an inclined training table in the position previously described.

For all participants, the start of CWI intervention occurred 5–8 min after the final set of exercise. This resulted in the 15 min post-exercise blood draw being taken in the tub 7–10 min after the start of the CWI intervention and the 30 and 60 min post-exercise blood draws were taken after sitting on the training table for 7–10 min. This study design was adopted at is allowed for stable body position

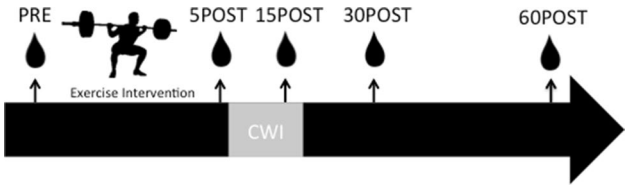

Fig. 1 Depiction of the sequence of events during a cold-water immersion (CWI) experimental session

and a homeostatic fluid balance to be reached before blood samples were taken (see Fig. 1).

Blood biomarkers

Prior to each experimental session, participants had a flexible 18-gauge Teflon catheter (Surflow, Terumo Medical Products, Somerset, NJ, USA) inserted into their antecubital vein. Venous blood samples were taken pre-exercise (PRE), and 5 (5POST), 15 (15POST), 30 (30POST), and 60 (60POST) min post-exercise. At each time-point, LA was measured in triplicate from a serum venous blood sample within 5 min of its collection using a lactate analyzer (Lactate Plus, Nova Medical, Waltham, MA, USA).

Coagulated serum samples were centrifuged (10 min, 2000×*g*, room temperature) and the separated serum was stored (− 80 °C) for measurements of T, IL-6, and TNFα via enzyme-linked immune-sorbent assays (ELISA). ELISAs were conducted according to the manufacturer's instructions (11-FTSHU-E01, Alpco, Salem NH and #501030, #589201, Cayman Chemical, Ann Arbor, MI, USA) and absorbances read on a SpectraMax M2 (Molecular Devices, Sunnyvale, CA, USA). Average intra-assay CVs for samples run in duplicate were < 17%. Biomarker concentrations were interpolated from best-fit standard curves (TNFα and IL-6: origin as *y*-intercept). TNFα standard curves were fit using a second-order polynomial (quadratic) equation, IL-6 curves were fit using a linear equations, and testosterone curves were fit using semi-log curves. The type of standard curve used for each variable was determined by comparing goodness of fit ($p > 0.05$, $R^2 > 0.9$) between linear, quadratic, 3- 4- and 5-parameter logistic, and semi-log equations (Graphpad Prism 7.0, La Jolla, CA, USA).

Statistical analyses

To determine if exercise intensity differed between resistance training bouts CR-10 was compared between conditions using a paired *t* test and LA was compared between conditions and over time using a 2 × 5 (intervention × time) repeated-measures analysis of variance (rANOVA). To account for the individual variations in baseline (PRE) T, IL-6, and TNFα, relative changes in concentration were compared between conditions and over time using a 2 × 4 (intervention × time) rANOVA. For each rANOVA, normality (Shapiro–Wilk) and sphericity (Mauchly's) assumptions were tested prior to the rANOVA and Greenhouse–Geisser corrections were made where appropriate and pairwise comparisons were measured using Bonferroni post hoc tests. As data for IL-6 and TNFα were not normally distributed and violated equal variance assumptions, a nonparametric Kruskal–Wallis test with Dunnett's post hoc tests was used to determine if significant differences were present. Significance was accepted at $p \leq 0.05$ and data are presented as mean and standard errors.

Results

The resistance-exercise protocol resulted in similar levels of perceived exertion (CR-10) in CWI (9.3 ± 1.2) and CON (8.8 ± 1.7) between conditions ($p = 0.177$) with reported 95% confidence intervals for both conditions falling between rating of very strong (7) to extremely strong (10) exertion. All participants were able to successfully replicate the number of repetition performed and load lifted on both days showing similar physical work between conditions. Measurements of LA throughout testing are shown in Fig. 2. The exercise intervention resulted in a significant increase in LA from PRE at all time-points for both conditions ($p = 0.000$–0.004). In addition, no main effect of condition was observed and no differences between conditions were observed at any time-point (15POST: $p = 0.481$, 30POST: $p = 0.080$, 60POST: $p = 0.124$).

Changes in T, IL-6, and TNFα after a bout of resistance exercise are presented in Fig. 3. A main effect of time was observed for T ($p < 0.001$) with significant elevation in

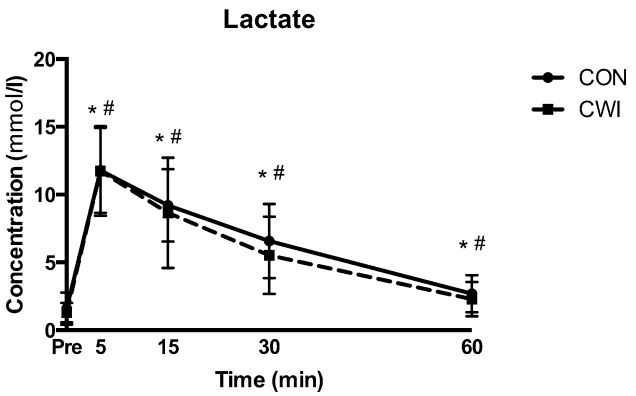

Fig. 2 Changes in blood lactate (LA) in response to a bout of resistance exercise in cold-water immersion (CWI) and control (CON) conditions. Absolute values (mean ± SD, mmol L^{-1}) are shown with differences from PRE values are indicated by asterisk for the CON condition and superscript number sign for the CWI condition ($p < 0.05$)

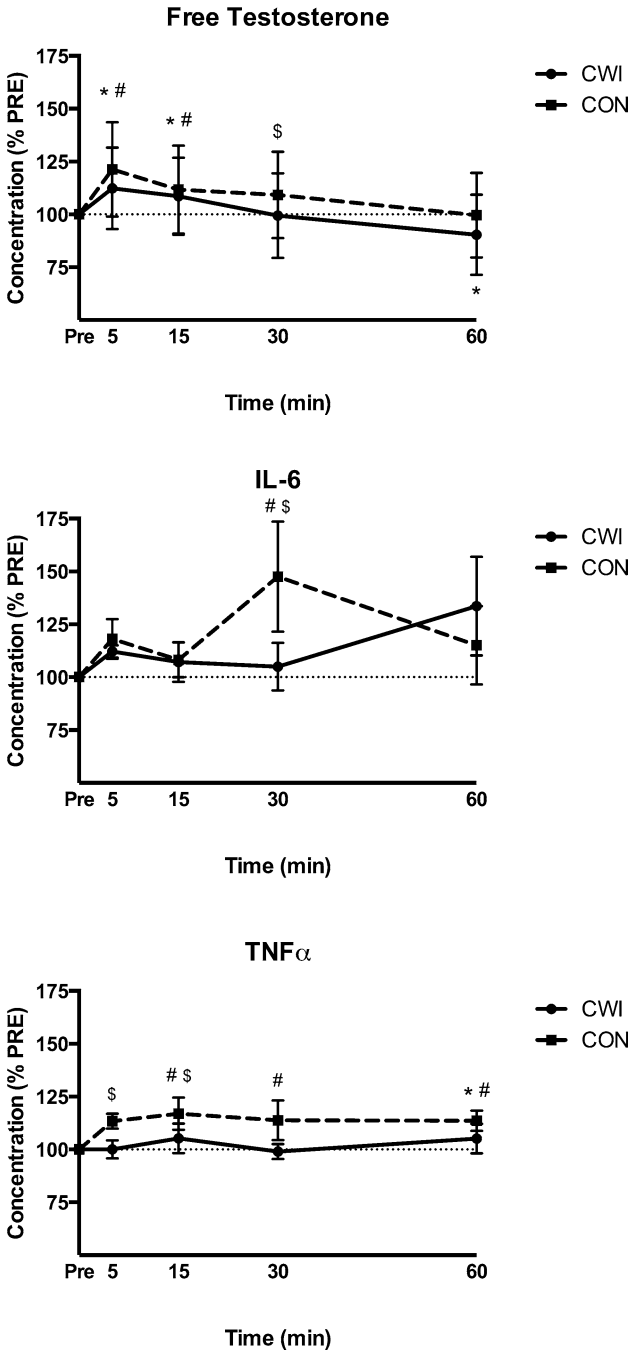

Fig. 3 Changes in free serum testosterone (T) interleukin-6 (IL-6) and tumor necrotic growth-factor (TNFα) concentrations after a bout of resistance exercise in cold-water immersion (CWI) and control (CON) conditions. Relative changes from pre-exercise (PRE) values are reported (Δ%, mean ± SE). A significant interaction effect of condition over time was observed for T ($p = 0.030$) and TNFα ($p = 0.023$) but not IL-6 ($p > 0.05$). Differences at individual time-points from PRE are indicated by asterisk for CON and number sign for the CWI, while differences between conditions are indicated by dollar sign ($p < 0.05$)

concentrations at 5POST and 15POST ($p = 0.006$). Post hoc tests revealed T concentrations were similar to PRE values in both conditions by 30POST and at 60POST concentrations dropped below PRE values in CWI but not CON. A significant interaction effect of condition and time was found for T ($p = 0.030$); however, the only time-point where a significant difference between conditions was observed 30POST ($p = 0.049$).

A main effect of time was also observed for IL-6 ($p = 0.046$), but post hoc tests revealed that the only condition and time-point in which IL-6 concentrations were significantly different from baseline measurements was in the CON group at 30POST. At 30POST, it was also observed that IL-6 concentrations were significantly greater in CON than CWI ($p = 0.041$).

A condition by time effect was observed for TNFα in CON ($p = 0.023$) where concentrations were significantly greater than PRE at 15POST, 30POST, and 60POST. While a condition by time effect was also observed for TNFα in CWI ($p = 0.027$), concentrations were only significantly greater than PRE at 60POST. When compared to CON lower TNFα, concentrations were observed in CWI at 5POST ($p = 0.012$) and 15POST ($p = 0.022$), but values were similar between conditions at 30POST ($p = 0.078$) and 60POST.

Discussion

In the present study, resistance-trained men completed two resistance-exercise bouts with identical workloads, while circulating T, IL-6, and TNFα concentrations were measured after participants underwent 15 min of CWI or passive recovery (CON). The exercise protocol elicited an acute T response where concentrations increased from PRE at 5POST and 15POST before returning to baseline values at 30POST in both conditions. However, despite a similar general trend, the magnitude of this response differed between conditions as indicated by a significant interaction effect of condition by time. Furthermore, at 30POST significantly less T was observed in CWI than CON and at 60POST concentrations in the CWI condition dropped below PRE. These results suggest that CWI suppresses the acute post-resistance exercise T response, which has been attributed to protein synthesis (Kraemer et al. 2017). This suppression is likely partially responsible for the altered mTOR pathway kinase activity previously observed by Roberts et al. (2017) due to the upstream role of T in this pathway (Morita et al. 2015; Wang and Proud 2006). When interpreting these results, it is of interest to note that, during 15POST, participants were still actively undergoing CWI (i.e. immersed in cold water) and it was not until the recovery periods after the cryotherapy (30POST and 60POST) that T response differed between the CWI and CON conditions.

While the present study found that the acute T response was suppressed after CWI, results from the present study only elucidate the response up to 60 min post-exercise. Because CWI resulted in lower T values at the final time-point compared to PRE, more research is needed to determine the duration of this suppression. In a study by Russell et al. (2017), T response was compared between whole-body cryotherapy, a form of cryotherapy in which super cold air (− 60 to − 120 °C) is delivered in a short period of time (30–120 s) via a cryochamber (Rose et al. 2017), to passive recovery after repeated 30 m sprints. In this study, the authors observed increased testosterone 2 and 24 h after exercise. Unfortunately, these results are difficult to compare to the present study as (1) the exercise protocol was not appropriate to elicit an increase in hypertrophy or strength in the target population, (2) chronic changes in testosterone may be related more to cumulative training stress and recovery than protein synthesis (Urhausen et al. 1995), and (3) little is known how the physiological response of CWI relates to whole-body cryotherapy (Abaidia et al. 2017). There is also some evidence that chronic use of CWI may alter resting T levels with studies showing both positive (Grasso et al. 2014) and negative (Juarez-Rojas et al. 2015) effects. Similar to the 24 h post-exercise measures reported by Russell et al. (2017), it is likely that chronic T indicates cumulative exercise stress and recovery rather than protein synthesis (Urhausen et al. 1995).

Similar to T, the acute IL-6 and TNFα response to resistance exercise were altered by CWI. In the present study, the exercise failed to elicit a significant increase in IL-6 from PRE at any time-points with the exception of 30POST. However, at this time-point, CON experienced an increase from PRE values that was significantly larger than that experienced during CWI. In comparisons, Peake et al. (2017) found that plasma IL-6 was elevated from baseline immediate post-exercise as well as 30 and 60 min post-exercise in both a CWI and an active recovery condition. Similarly, Roberts et al. (2014) found an increase in plasma IL-6 after both CWI and an active recovery condition at 15 and 60 min post-exercise. Differences between these and the present study may be attributed to the resistance training status of the participants. Specifically, in the present study, participants were resistance-trained (1RM:BM: 1.65 ± 0.15), while participants in Peake et al. (2017) and Roberts et al. (2014) were described only as physically active.

While neither Peake et al. (2017) nor Roberts et al. (2014) observed any between condition differences in IL-6 response during the time period utilized in the present study (60 min post-exercise), both authors did observed a differing IL-6 response at 120 min post-exercise with differing conclusions. Roberts et al. (2014) observed significantly greater plasma IL-6 concentrations in the active recovery condition than the CWI condition at 120 min post-exercise, while Peake et al. (2017) observed a return to pre-exercise IL-6 values in the CWI condition and sustained elevated values in the active recovery condition. As the present study did not take any plasma samples during this time period, the acute effects of CWI on IL-6 outside of this time period remain in question.

TNFα is another cytokine that can be suppressed by cryotherapy (Peake et al. 2017; Ziemann et al. 2012) and is involved in the mTOR pathway (Morita et al. 2015). While the previous works have shown CWI can suppress the TNFα response, 2 h post-resistance exercise, the present study is the first to examine differences within an hour of exercise. In the present study, TNFα concentrations were greater in CON than CWI at 5POST and 15POST, and at 30POST, TNFα concentrations were elevated in CON but not CWI. While these results suggest that TNFα response is acutely suppressed by CWI, caution should be taken when interpreting these results as significant differences between conditions were observed a 5POST, which is prior to the CWI intervention.

Conclusions

Exposure to circulating 15 °C water for 15 min after an intense bout of resistance exercise resulted in a blunted and delayed acute (within 60 min) testosterone and cytokine response when compared to a passive recovery in a thermoneutral room. These results suggest that the previous observations of attenuated strength and hypertrophy seen after CWI is performed acutely after resistance exercise may be due in part to an impaired testosterone response due to decreased biological availability testosterone.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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