Postexercise Muscle Cooling Enhances Gene Expression of PGC-1α

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ABSTRACT

IHSAN, M., G. WATSON, H. C. CHOO, P. LEWANDOWSKI, A. PAPAZZO, D. CAMERON-SMITH, AND C. R. ABBISS. Postexercise Muscle Cooling Enhances Gene Expression of PGC-1α. *Med. Sci. Sports Exerc.*, Vol. 46, No. 10, pp. 1900–1907, 2014. **Purpose**: This study aimed to investigate the influence of localized muscle cooling on postexercise vascular, metabolic, and mitochondrial-related gene expression. **Methods**: Nine physically active males performed 30 min of continuous running at 70% of their maximal aerobic velocity, followed by intermittent running to exhaustion at 100% maximal aerobic velocity. After exercise, subjects immersed one leg in a cold water bath (10°C, COLD) to the level of their gluteal fold for 15 min. The contralateral leg remained outside the water bath and served as control (CON). Core body temperature was monitored throughout the experiment, whereas muscle biopsies and muscle temperature (T_m) measurements were obtained from the vastus lateralis before exercise (PRE), immediately postexercise (POST-EX, T_m only), immediately after cooling, and 3 h postexercise (POST-3H). **Results**: Exercise significantly increased core body temperature (PRE, 37.1°C ± 0.4°C vs POST-EX, 39.3°C ± 0.5°C, $P < 0.001$) and T_m in both CON (PRE, 33.9°C ± 0.7°C vs POST-EX, 39.1°C ± 0.5°C) and COLD legs (PRE, 34.2°C ± 0.9°C vs POST-EX, 39.4°C ± 0.3°C), respectively ($P < 0.001$). After cooling, T_m was significantly lower in COLD (28.9°C ± 2.3°C vs 37.0°C ± 0.8°C, $P < 0.001$) whereas PGC-1α messenger RNA expression was significantly higher in COLD at POST-3H ($P = 0.014$). Significant time effects were evident for changes in vascular endothelial growth factor ($P = 0.038$) and neuronal nitric oxide synthase ($P = 0.019$) expression. However, no significant condition effects between COLD and CON were evident for changes in both vascular endothelial growth factor and neuronal nitric oxide synthase expressions. **Conclusions**: These data indicate that an acute postexercise cooling intervention enhances the gene expression of PGC-1α and may therefore provide a valuable strategy to enhance exercise-induced mitochondrial biogenesis. **Key Words:** EXERCISE RECOVERY, MUSCLE AEROBIC ADAPTATIONS, COLD WATER IMMERSION, MITOCHONDRIAL BIOGENESIS, VASCULAR ENDOTHELIAL GROWTH FACTOR, NONSHIVERING THERMOGENESIS

Endurance training results in a broad range of important skeletal muscle adaptations that improve aerobic capacity, including increased mitochondrial content (12) and capillary density (6) and improved conduit and microvascular function (17,34). Although the mechanisms underlying these adaptations are not completely understood, it is well accepted that the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is a key regulator of mitochondrial biogenesis, and vascular and metabolic adaptations to exercise (45). Specifically, PGC-1α has been shown to regulate the expression of the cellular glucose transporter 4 (GLUT4) and the angiogenic and arteriogenic signaling protein vascular endothelial growth factor (VEGF) (4,23). Expression of PGC-1α and subsequent downstream regulators seems to be influenced by nitric oxide (NO) (27). Indeed, in rodent and cell culture models, treatment with an NO synthase (NOS) inhibitor or NO donor has been shown to suppress or enhance the gene expression of VEGF and GLUT4, respectively (9,19). These results highlight NO and PGC-1α as principal regulators of the skeletal muscle mitochondrial, vascular, and metabolic adaptations to exercise.

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The production of metabolic heat during exercise seems to have a role in muscle oxidative phenotype transformations (10,11,20,26). For instance, evidence of mitochondrial biogenesis, in line with concomitant increases in PGC-1α activity and expression, was evident in muscle cell cultures exposed to heat (20). Moreover, heat exposure has also been shown to decrease resting blood glucose concentration and enhance arterial adaptations in humans (11,26), possibly via an increase in endothelial NOS (eNOS) activity and expression (10). In contrast, *in vivo* data from humans showed attenuated PGC-1α messenger RNA (mRNA) expression when postexercise recovery (3 h at 33°C) was undertaken in the heat (39). Interestingly, aerobic phenotype adaptations may also be induced by cold exposures. Indeed, PGC-1α is powerfully induced in response to cold, where it is implicated in the regulation of adaptive (i.e., nonshivering) thermogenesis (33,44). In addition, up-regulation of eNOS and VEGF, indicating enhanced vascular adaptations, has also been demonstrated after cold exposure in brown adipose tissue (7,15). Collectively, these data imply that although exercise-induced metabolic heat production may contribute to muscle oxidative adaptations, additional heat exposure during recovery seems to be detrimental. In this regard, cold exposure during recovery may serve as a potential strategy to enhance muscle aerobic adaptations to exercise.

Postexercise cold water immersion (CWI) is an effective method for rapidly reducing body temperatures (29), muscle blood perfusion, and metabolic activity (14). As such, this recovery strategy is widely used among athletes of all levels to ameliorate hyperthermia-induced fatigue (30) and in the treatment of exercise-induced muscle damage (42). Indeed, postexercise CWI has shown to maintain subsequent exercise performance (30), preserve day-to-day performance (41), and, in some (42) but not all cases (31), attenuate the increase in indirect markers of muscle damage. Although studies investigating the effect of a recovery-based CWI intervention on muscle adaptations are currently lacking, Slivka et al. (38,39) reported significant elevations in PGC-1α mRNA expression when a 3- to 4-h recovery period was undertaken in cold (7°C) compared with those in room (20°C) temperature. However, the increase in PGC-1α after the prolonged cold exposure was accompanied by a significant increase in whole-body oxygen consumption and shivering thermogenesis, and hence does not isolate the effect of reduced tissue temperature *per se* on skeletal muscle adaptations. Indeed, minimal or unextended shivering thermogenesis is more likely during typical postexercise CWI interventions, which are generally performed at 10°C–15°C for 10–20 min (14,29, 41,42). Although existing evidence indicates that both heat and cold exposure *per se* activate skeletal muscle mitochondrial biogenesis and angiogenesis, it is currently unknown how rapidly reducing postexercise tissue temperatures via cooling interventions may subsequently influence these adaptations. Therefore, the purpose of this investigation was to examine the effects of acute postexercise cooling on the expression of genes related to mitochondrial biogenesis (PGC-1α, NOS) and their downstream targets mediating vascular (VEGF) and metabolic adaptations (GLUT4).

METHODS

Subjects

Nine physically active, healthy males (mean ± SD: age, 25.8 ± 3.9 yr; height, 174 ± 7 cm; mass, 71.8 ± 9.2 kg; maximal oxygen consumption ($\dot{V}O_{2max}$), 52.0 ± 4.6 mL·kg^{-1}·min^{-1}) were recruited for this study. Subjects had been participating in aerobic exercises for 3–4 h·wk^{-1} for at least a year at the time this study was conducted. Subjects were not under any medication, had no history of lower limb musculoskeletal injuries, and were told to refrain from exercise, alcohol, and caffeine for at least 48 h before the testing sessions. They were fully informed of the requirements and risks associated with the study, and a written informed consent was obtained before participation. This study was approved by the Edith Cowan University human research ethics committee.

Experimental Design

Incremental running test. Each participant attended the laboratory on two separate occasions separated by 9–14 d. On their first visit, subjects performed an incremental running test on a motorized treadmill (Trackmaster; JAS Fitness Systems) for the determination of their maximal running velocity (V_{max}) and $\dot{V}O_{2max}$. The test commenced at an initial speed of 10 km·h^{-1} and increased by 1 km·h^{-1} every 2 min until volitional exhaustion. The gradient of the treadmill was maintained at 1%. Heart rate (HR) (S610, Polar, Finland) and gas exchange (TrueOne, ParvoMedics) were continuously recorded throughout the test. Before all tests, the gas analyzer and the ventilometer were calibrated using gases of known concentrations and a 3-L syringe (5530 series; Hans Rudolph, Inc.), respectively. Maximal oxygen uptake was recorded as the highest value attained in any 30-s average, and V_{max} was calculated using the following equation: $V_{max} = V_f + (t/120)$ where V_f is the velocity achieved during the last completed stage in kilometers per hour and t is the time of the incomplete stage in seconds. After the incremental test and sufficient recovery, subjects were fully familiarized with the exercise and the CWI protocol that was to be undertaken on their subsequent visit.

Experimental trial. The experimental trial commenced in the morning between 7 and 9 a.m. where subjects arrived having ingested 500 mL of water and a standardized meal consisting of 300 kcal (80% CHO, 14% protein, and 6% fat) 2.5 h before the trial. The exercise and cooling protocols used in the present study were similar to those used in previous work conducted in our laboratory (13,14). Briefly, subjects performed 30 min of continuous running at 70% of their V_{max}, followed by intermittent running to exhaustion at 100% of their V_{max}. The work and rest durations during the intervals were 30 s and 15 s, respectively, with a 1-min rest period between the continuous and intermittent bouts.

Intermittent protocols of such nature allow longer exercise time at $\dot{V}O_{2max}$ (2), which is an important stimulus for up-regulating the genes of interests (22,28). Moreover, previous studies have reported good test–retest reliability (coefficient of variation, 4%–6%) and no significant differences between legs for mean muscle oxygenation and blood volume changes during an identical intermittent treadmill protocol (13,14). As such, the exercise protocol used in the current study is likely to have resulted in similar physiological stimuli in both the intervention and control limbs. Within 2 min after the cessation of exercise, subjects immersed one leg (COLD) to the level of their gluteal fold into a plastic water bath (47 × 41 × 87 cm) maintained at 9.8°C ± 0.2°C for 15 min while their contralateral leg rested outside the water tank. The contralateral leg received no cooling treatment and thus served as control (CON). The cooled limb was randomized between subjects' dominant and nondominant leg. Although recovery CWI usually involves whole-body or waist-deep immersions, we used this one-legged protocol to control for shivering thermogenesis, which could possibly influence the gene expression data (38,39). Moreover, this protocol has minimal influence on the natural decline in postexercise core body temperature (T_c) (14), hence isolating the effects of a lowered muscle temperature (T_m) *per se* on the changes in postexercise gene expression.

Vastus lateralis T_m were taken from both legs (CON and COLD) before exercise (PRE), within 1 min after exercise (POST-EX) and immersion (POST-COLD), and 3 h postexercise (POST-3H). The T_m measurement order between CON and COLD legs was randomized between subjects and obtained within 15 s for each leg (as will be described later). In addition, muscle tissue samples were obtained from the vastus lateralis PRE, POST-COLD, and POST-3H. PRE biopsies were taken from one leg, which was randomized between CON and COLD. POST-COLD and POST-3H biopsies were taken from both legs, approximately 5 min apart, with the order randomized between CON and COLD. HR and T_c were continuously recorded throughout the experimental protocol. Subjects rested in the laboratory throughout the 3-h recovery period, during which they consumed no food but were allowed water *ad libitum*. All experimental sessions were conducted in an environmental chamber controlled at 23.3°C ± 1.1°C and 35% ± 6% relative humidity.

Experimental Procedures and Measurement

T_m and T_c measurements. T_c was measured using a disposable rectal thermometer (Monatherm 400 series; Mallinckrodt Medical) inserted approximately 12 cm past the anal sphincter. The thermometer was connected to a data logger (Squirrel SQ2020; Grant Instruments, Cambridge, United Kingdom), and T_c measurements were subsequently sampled at 1 Hz throughout the experiment. Because of the sensitive nature of rectal temperature measurements, T_c in three of our subjects was determined via ingestible temperature measurement pills (CorTemp; HQ, Inc.) coupled with a handheld data logger (HT150001 CorTemp; HQ Inc.). On these occasions, subjects ingested the pill at least 5 h before arriving at the laboratory. Measurement of T_c via telemetric pills is systematically biased by −0.15°C, compared with rectal temperature measurements (8). This bias is negligible, considering that having used a one-legged study design, there is no need for between-trial comparisons for T_c measurements and they are presented here for descriptive purposes only. The T_m of the vastus lateralis was measured at a 3-cm depth using a thermometer (TH; Physitemp Instruments, Inc.) connected to a sterile 26-gauge needle probe (MT-26/4; Physitemp Instruments, Inc.). All T_m measurements were performed under topical anesthesia (5% lidocaine) standardized at the midpoint of the vastus lateralis, with subsequent incisions performed within a 1-cm radius relative to the initial incision. Once the needle was inserted into the muscle, reading was taken after the attainment of a steady T_m value, which took approximately 5 s. The overall time involved in the procedure (i.e., needle insertion, reading, and withdrawal) took no longer than 15 s. At the end of each experimental session, needle probes were checked against a mercury thermometer (Model 526-10942; WIKA, New South Wales, Australia) in a 34°C water bath and subsequently sterilized before further use.

Muscle biopsy. Muscle samples were extracted using a disposable, spring-loaded microbiopsy system (MAX-CORE®, Bard Biopsy Systems). After the application of topical anesthesia (5% lidocaine) around the sampling region, a 13-gauge cannula was inserted 3 cm into the belly of the vastus lateralis. A 14-gauge biopsy needle was inserted into the cannula, and 2–3 muscle samples were subsequently extracted per biopsy. Initial biopsy was performed 5 cm distal to the midpoint of the vastus lateralis, with subsequent biopsies performed within a 1.5-cm radius relative to the initial biopsy site. The tissue samples were immediately frozen in liquid nitrogen and stored in a –80°C freezer for later analysis.

Gene expression. Total RNA was extracted from 10 mg of frozen muscle using TRI reagent (Astral Scientific, New South Wales, Australia) according to the manufacturer's specification. The total RNA concentration was determined by A260, and purity of the RNA, by A260/A280 measurement. One microgram of total RNA was reverse-transcribed into complementary DNA (cDNA) using avian myeloblastosis virus reverse transcriptase first strand cDNA synthesis kit according to the manufacturer's protocol (Marligen Biosciences, Australia). Real-time polymerase chain reaction was performed using a Bio-Rad IQ5 detection system, with reactions performed using SYBR Green Supermix (Bio-Rad, New South Wales, Australia). Primers (see table, Supplemental Digital Content 1, PCR primers for gene expression, http://links.lww.com/MSS/A380) were designed using Primer 3 and obtained from GeneWorks (Hindmarsh, Australia). The amplification of cDNA samples (0.5 ± 0.009 ng) was carried out using IQ SYBR green™ following the manufacturer's protocols (BioRad, New South Wales, Australia). Fluorescent

emission data were captured, and mRNA levels were analyzed using the critical threshold value (36). Thermal cycling and fluorescence detection were conducted using the BioRad IQ5 sequence detection system (BioRad, New South Wales, Australia). As previously described (21,28,40), the mRNA of each gene was normalized to its cDNA concentration determined with OliGreen (Invitrogen, Melbourne, Australia). This method bypasses many problems associated with normalizing to "housekeeping genes" and hence serves as a robust and suitable alternative method of mRNA normalization (21).

Statistical Analysis

Data distribution was assessed using the Shapiro–Wilk test, which demonstrated no deviations from normality in all variables. Changes in mRNA expression and T_m were analyzed using a two-way mixed model ANOVA (condition–time), where the within-subject factor was time and the between-subject factor was condition (CON vs COLD). Changes in T_c were analyzed using a one-way repeated-measures ANOVA. Where significant effects were evident, secondary analysis using Fisher least significant difference tests were undertaken to locate the differences. All statistical analyses were performed using SPSS version 19 (IBM, SPSS, Inc.). Significance level was accepted as $P < 0.05$, and all data are presented as mean ± SD.

RESULTS

Exercise. Mean running velocity at 70% V_{max} and V_{max} were 10.2 ± 0.7 km·h^{-1} and 14.6 ± 0.9 km·h^{-1}, respectively. Subjects managed to perform 11.4 ± 8.2 intervals before exhaustion, corresponding to an intermittent running time, including rest intervals of 8.6 ± 6.1 min and total exercise time of 38.6 ± 6.1 min. Mean HR during the continuous and intermittent run was 88% ± 3% and 95% ± 2% of their HR_{max}, respectively.

Changes in T_c and T_m. T_c at the end of exercise and after cooling was significantly higher compared with that at rest ($P < 0.001$) but returned to PRE values at POST-3H ($P = 0.081$, Fig. 1A). Significant main effects for time, condition, and interaction were noted for changes in T_m (Fig. 1B) during exercise and recovery ($P < 0.001$). Exercise significantly elevated T_m in both CON and COLD ($P < 0.001$), with no significant differences observed between conditions ($P = 0.159$). In contrast, T_m in COLD was significantly lower than that in CON after 15 min of COLD ($P < 0.001$). No significant differences in T_m were observed between conditions at POST-3H ($P = 0.059$).

Changes in gene expression. Significant time ($P = 0.002$), condition ($P = 0.010$), and interaction ($P = 0.019$) effects were observed for changes in PGC-1α mRNA expression (Fig. 2A). Specifically, PGC-1α mRNA expression at POST-3H was significantly greater in COLD compared with that in CON ($P = 0.014$) and in PRE values ($P < 0.001$). Significant time effects ($P = 0.038$) but not condition ($P = 0.279$) and interaction ($P = 0.299$) effects were observed for VEGF mRNA (Fig. 2B). Pairwise comparisons revealed that compared with baseline values, VEGF mRNA at POST-3H increased significantly in the COLD ($P = 0.036$) but did not significantly change in the CON condition ($P = 0.402$). Significant time effects ($P = 0.019$) with no condition ($P = 0.080$) or interaction ($P = 0.162$) effects were observed for neuronal NOS (nNOS) mRNA expression (Fig. 3A). Compared with the PRE value, nNOS mRNA expression at POST-3H was significantly higher in COLD ($P = 0.008$) but not in CON ($P = 0.390$). Changes in inducible NOS (Fig. 3C) were insignificant for time ($P = 0.055$), condition ($P = 0.319$), and interaction ($P = 0.458$). Likewise, no significant main effects were observed for the changes in eNOS (Fig. 3B), GLUT4 and cytochrome oxidase 4 mRNA (Fig. 4) expression.

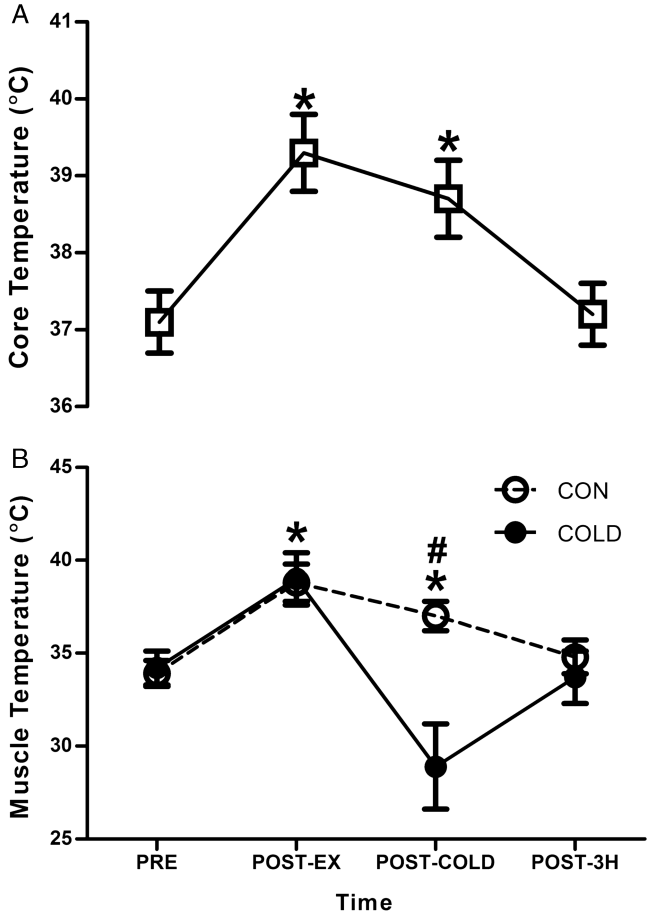

FIGURE 1—T_c (A) and vastus lateralis T_m (B) at PRE, POST-EX, after 15 min of cooling at 10°C (POST-COLD), and POST-3H. *Significantly different from PRE in CON and COLD ($P < 0.001$). #Significantly different between CON and COLD ($P < 0.001$).

DISCUSSION

To the best of our knowledge, this is the first investigation to examine the acute effects of a postexercise cooling

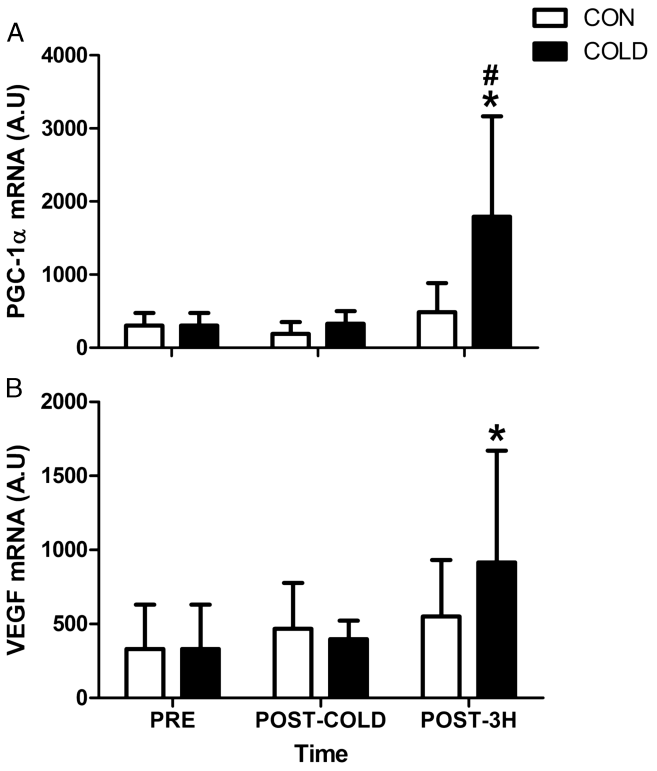

FIGURE 2—Gene expression of PGC-1α (A) and VEGF (B) at PRE, after 15 min of cooling at 10°C (POST-COLD), and at POST-3H. Changes in mRNA content are presented in arbitrary units (AU). *Significantly different from PRE in COLD ($P < 0.01$). #Significantly different between CON and COLD ($P < 0.05$).

intervention on the key regulators of mitochondrial biogenesis (PGC-1α and NOS) and their downstream regulators related to vascular adaptations (VEGF) and metabolic function (GLUT4). Despite CWI being a popular postexercise recovery intervention, it is currently unknown how this modality might influence skeletal muscle adaptations to exercise, given that both heat and cold exposure *per se* have previously been shown to enhance PGC-1α and NOS expressions in rodent and cell culture models (3,10,15,33). Cooling significantly lowered postexercise T_m. This change was associated with a significant increase in PGC-1α mRNA expression in COLD compared with that in CON. However, associated targets VEGF and nNOS only demonstrated significant changes from baseline (i.e., time effects), with no evident significant changes between conditions. As such, the present data indicate that localized postexercise muscle cooling enhances PGC-1α and hence, possibly mitochondrial biogenesis. However, its influence on VEGF and nNOS expression and associated functional adaptations warrant further research.

Although heat exposure has been shown to induce mitochondrial biogenesis and enhance vascular adaptations in cell cultures and humans (10,11,20,26), extended heat exposure after exercise (3 h at 33°C) reduced PGC-1α mRNA expression (39). In this regard, postexercise cold exposure could potentially increase the expression of PGC-1α. Indeed, our results indicate that postexercise cooling enhanced PGC-1α mRNA expression (Fig. 2A). This is consistent with the recent findings of Slivka et al. (38,39) who observed increased mRNA expression of PGC-1α after 3–4 h of passive recovery undertaken in a cold (7°C) environment. However, the studies by Slivka et al. (38,39) does not verify if the increase in PGC-1α was a result of cold exposure *per se* or due to the significant increase in whole-body oxygen consumption as a result of cold exposure. PGC-1α under the latter circumstances may be induced by other signaling pathways such as Ca^{2+} and/or by adenosine monophosphate-activated protein kinase (AMPK) mechanisms. Conversely, our protocol involved cooling the leg from the gluteal fold downwards, which did not result in shivering thermogenesis. Furthermore, a recent study by Ihsan et al. (14) demonstrated reduced muscle energy demand after an identical cooling protocol as used in this study. In this regard, we have demonstrated that skeletal

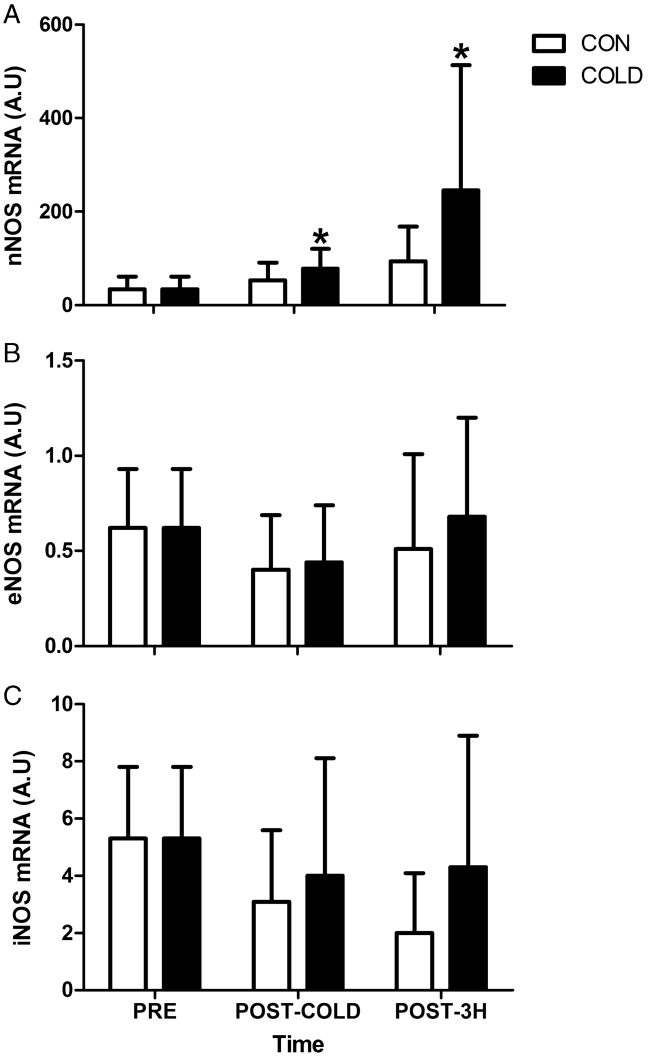

FIGURE 3—Gene expression of nNOS (A), eNOS (B), and inducible NOS (C) at PRE, after 15 min of CWI at 10°C (POST-CWI), and at POST-3H. Changes in mRNA content are presented in arbitrary units (AU). *Significantly different from PRE in CWI.

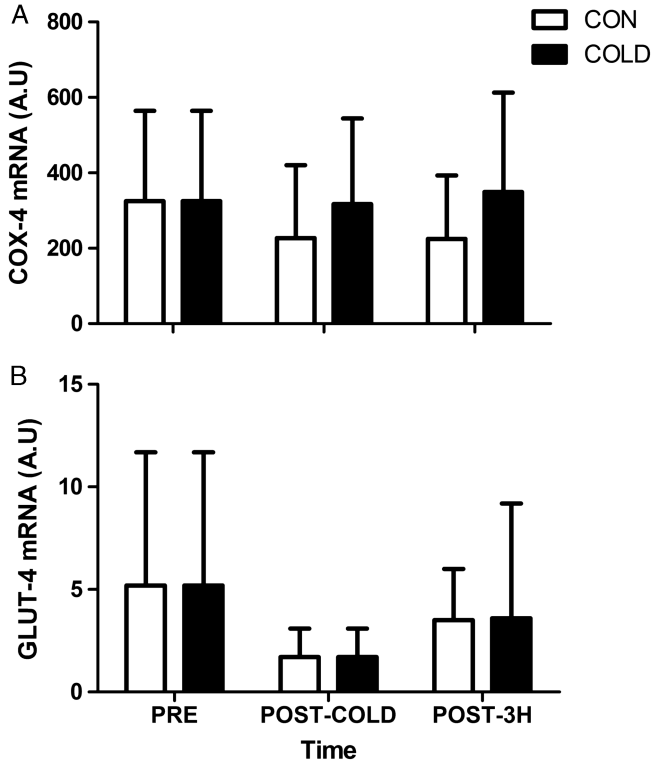

FIGURE 4—Gene expression of cytochrome oxidase 4 (A) and GLUT4 (B) at PRE, after 15 min of cooling at 10°C (POST-COLD), and at POST-3H. Changes in mRNA content are presented in arbitrary units (AU).

muscle PGC-1α mRNA expression may be elevated in response to cold *per se* in the absence of increased local/muscular metabolic demand.

We hypothesize that the enhanced PGC-1α expression observed in the present study could possibly be due to cold-induced β-adrenergic stimulation. Noradrenaline release and subsequent adrenergic stimulation after localized (nonshivering) cooling have been observed in humans (37) and have previously been shown to induce PGC-1α expression in cell cultures and rodents (25,33,44). Indeed, treatment with β-adrenergic agonist increased PGC-1α expression in brown fat (33) and skeletal muscles of healthy but not β-deficient mice (25). Likewise, treatment with β-antagonist propranolol abolished both β-agonists and exercise-induced increases in muscle PGC-1α (25). Cold-induced increases in PGC-1α have been suggested to regulate adaptive (i.e., nonshivering) thermogenesis by coordinating mitochondrial biogenesis and inducing the expression of mitochondrial uncoupling proteins in brown fat and skeletal muscles (33,44). It therefore seems possible that the enhanced PGC-1α expression observed in the present study may be associated with β-adrenergic stimulation initiating adaptive thermogenesis.

NO production and bioavailability has been shown to be essential in the expression of PGC-1α and subsequent mitochondrial biogenesis (27). Pharmacological inhibition of NOS activity has been shown to attenuate vascular function (16) and impair VEGF expression and capillary proliferation (9,24), indicating a functional and signaling role for NO in skeletal muscle vasculature. Moreover, NO signaling has been shown to induce GLUT4 expression and facilitate GLUT4 translocation and muscle glucose uptake (1,19,35). Given that training-induced increases in NOS protein expression increases NO production (22), we were interested to elucidate the effects of postexercise cooling on this important factor. We did not see any condition effects for the three major NOS isoforms expressed in mammalian skeletal muscle (Fig. 3). However, time effects, indicating partial up-regulation, were evident for changes in nNOS in the COLD condition (Fig. 3A). These findings are surprising given that PGC-1α, a downstream target of NO (27) demonstrated significant condition effects after COLD treatment (Fig. 2A). Moreover, cold-induced adrenergic activation of eNOS has been previously demonstrated in brown adipose tissue (15). Part of this discrepancy in results may be related to AMPK activation. A recent by study Lira et al. (18) showed that AMPK was essential in NO-mediated PGC-1α expression. Moreover, it was shown that although AMPK was downstream of NO, a feedback system between AMPK and NO was evident, as pharmacological activation of AMPK resulted in increased NO production in myotubes (18). It is well known that AMPK is readily activated by reductions in cellular energy availability (43). In contrast, we have previously shown reduced muscle metabolic demand after a cooling protocol identical to the present study (14). Taken together, although cold-induced β-adrenergic mechanisms might have up-regulated PGC-1α expression, concomitant attenuation in AMPK activation, resulting in an impaired AMPK-mediated NOS expression, might have been possible.

The growth factor VEGF is strongly implicated in the regulation of vascular arteriogenic and angiogenic processes (32). β-adrenergic stimulation induces VEGF expression via PGC-1α and the orphan nuclear receptor pathway (ERRα) (4), whereas pharmacological inhibition of NOS activity attenuates VEGF expression (9). Collectively, this indicates that VEGF is a downstream target for both PGC-1α and NO. Similar to changes in nNOS expression, we observed an effect for VEGF in the COLD-treated leg, with no significant differences between conditions (Fig. 2B). This indicates that an acute postexercise cooling treatment is insufficient to stimulate the VEGF gene response despite activating the upstream PGC-1α gene. This may be due to several putative mechanisms where firstly, the lack of VEGF expression might be related to upstream changes in nNOS. We hypothesized that the reduction in muscle metabolic demand after cooling (14) might have attenuated the activation of AMPK, which in turn might have attenuated NOS expression (18). This same pathway might have attenuated VEGF expression as well because VEGF is downstream of NO (9). Alternatively, Slivka et al. (38) demonstrated increased PGC-1α but reduced ERRα expression after 4 h of postexercise recovery undertaken in cold temperatures (7°C air). The expression of ERRα is necessary in PGC-1α–mediated expression of VEGF (4) and, if attenuated by the COLD

intervention, could explain the lack of VEGF activation in the present study. Nevertheless, we acknowledge that the mechanisms activating the PGC1–VEGF axis may be different in the study by Slivka et al. (38) and the present study because of differences in cooling modality (whole-body cold air exposure at 7°C vs localized CWI at 10°C) and the duration (4 h vs 15 min) used. Further investigations on the effect of repeated cooling stimulus on VEGF response are certainly warranted.

GLUT4 is a downstream target of both PGC-1α and NO (19,23) and has been previously shown to increase after exercise (35). Considering that PGC-1α but not NOS was up-regulated after cooling, it would have been interesting to note subsequent downstream effects on GLUT4 gene expression. However, neither exercise nor cooling influenced GLUT4 expression in the present study. We speculate that the lack of an exercise effect on GLUT4 expression may be due to the CHO-rich meal that our subjects consumed before the trial. Indeed, glucose ingestion has been shown to depress exercise-induced mRNA expression of several metabolic genes, including GLUT4 (5). Although we speculate that this could have interfered with our GLUT4 data, further research on postexercise cooling on metabolic gene expression is warranted

In conclusion, we investigated the acute effects of postexercise cooling on several key genes related to mitochondrial biogenesis, vascular adaptations, and metabolic function. Despite CWI being a popular postexercise recovery modality, there was little evidence to suggest how this intervention might influence muscle adaptations to exercise. The present study, for the first time, demonstrates that postexercise cooling of the muscles enhances exercise-induced mRNA expression of PGC-1α and, hence, possibly mitochondrial biogenesis. However, subsequent effects on nNOS and VEGF expressions are less clear and certainly warrant further investigation. We acknowledge some limitations in transferring our result to a highly trained athletic population. Moreover, mRNA expression is not necessarily reflective of functional new steady-state protein content. As such, further research examining the long-term effects of using this recovery modality on muscle aerobic function, vascular adaptations, and exercise performance is warranted.

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The authors have no conflict of interest.

The results of the present study do not constitute endorsement of American College of Sports Medicine.

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