A comparison of PGC-1α mRNA and protein expression in response to one week endurance training on alternate days or consecutive four days

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<td>Complete List of Authors:</td>
<td>Huang, Li-Ping; Tianjin University of Sport, Department of Health and Exercise Science</td>
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<td>Yao, Min; Tianjin University of Sport, Department of Health and Exercise Science</td>
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<td>Wang, Ya-Li; Tianjin University of Sport, Department of Health and Exercise Science</td>
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<td>Davie, Allan; Southern Cross University, School of Health and Human Science</td>
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<td>Zhou, Shi; Southern Cross University, School of Health and Human Sciences</td>
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Authors:
Li-Ping Huang 1, Min Yao 1, Ya-Li Wang 1, Allan Davie 2, Shi Zhou 2

Title:
A comparison of PGC-1α mRNA and protein expression in response to one week endurance training on alternate days or consecutive four days

Affiliations:
1 Department of Health and Exercise Science, Tianjin University of Sport, China.
2 School of Health and Human Sciences, Southern Cross University, Australia.

Corresponding author:
Professor Shi Zhou
School of Health and Human Sciences
Southern Cross University
P.O. Box 157
Lismore, NSW 2480
Australia

Email: shi.zhou@scu.edu.au
Telephone: +61 2 66203991
Fax: +61 2 66269583
Abstract

To understand the molecular mechanisms of adaptation to different training protocols, this study compared the effects of four sessions of 90 minutes treadmill exercise on alternate days or consecutive days in one week, on mRNA and protein expression of PGC-1α in rat gastrocnemius muscle. The mRNA significantly increased by 25.8 folds after alternate-day and 10.1 folds after consecutive-day training, while the protein showed no significant cumulative effect, 1.5-1.7 folds above baseline, in the two protocols.

Keywords:
PGC-1α, mRNA, protein, gastrocnemius, endurance training, recovery
Introduction

Adaptations to training represent the cumulative effect of repeated sessions of exercise and are highly specific to the exercise mode, intensity, duration, and frequency (Egan & Zierath 2013). At molecular level, adaptations are thought to be due to the cumulative effects of transient changes in mRNA transcripts and the proteins they encode following each training session (Bartlett et al. 2012; Hawley 2009). The recovery intervals between exercise sessions can influence such cumulative effects and subsequently the adaptations to training. There have been reports that compared the effects of training on consecutive days and non-consecutive days on physical performance (Gross et al. 2007), however the expressions of mRNA and protein for selected biomarkers have only been examined for different training protocols separately (Egan et al. 2013; Perry et al. 2010).

The peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) is a transcriptional coactivator and interacts with several nuclear transcription factors (Kang & Ji 2012). PGC-1α has been considered as a master regulator of mitochondrial biogenesis (Konopka & Harber 2014). It has been hypothesised that PGC-1α is a critical mediator of the muscle adaptive response to training (Little et al. 2011).

The aim of this pilot study was to compare the effects of one week endurance treadmill training (four sessions) between two commonly used training regimes: training on alternate days and training on consecutive days, on the expression of PGC-1α mRNA and protein.
Methods

Fifty-four male Sprague-Dawley rats, with age of 6-8 weeks and body mass (mean ±SD) 280 ±10 grams, were randomly divided into nine groups (n = 6 each). The research design and protocol for each group are presented in Table 1.

All animals were housed in an environment with temperature of 20-25ºC, relative humidity of 55-65%, lights 12 h on/12 h off, and adequate ventilation; and had access to normal commercial rodent feed and water *ad libitum*. The animals were euthanized with intraperitoneal injection of sodium pentobarbitone, one hour after the final training session, except that the CA0 and C7 groups were euthanized on the 7th day. The gastrocnemius muscle was dissected out and stored at -80ºC before analysis.

The mRNA expression were determined using fluorescent real-time polymerase chain reaction (RT-PCR) technique, following the instructions of Platinum® SYBR® Green qPCR SuperMix-UDG kit (Invitrogen™). Total RNA was extracted from muscle tissue using the standard Trizol RNA isolation method. The quality of RNA was checked using gel electrophoresis (3 µL RNA, clear separations at 28 s, 18 s, and 5 s. Sub-Cell® GT Agarose Gel Electrophoresis, BIO-RAD, USA) and ultraviolet spectrophotometer (DU800, Beckman, USA) at 260/280 nm (OD within 1.6-2.0). Then, reverse transcription of RNA was carried out using the RevertAid First Strand cDNA Synthesis Kit (Fermentas). The quantity of RNA was calculated from the 260 nm OD value. PCR (PE-9600, PE-Centus Co., USA) amplification was carried out in a total reaction volume of 25 µL, including 12.5 µL PCR buffer (Platinum® SYBR® Green qPCR SuperMix-UDG), 0.5 µL ROX dye correction, 0.5 µL PCR forward primer (10 PM), 0.5 µL PCR reverse primer (10 PM), 1.0 µL cDNA
template (×0.1) and 10.0 µL double distilled H$_2$O. β-actin was used as the internal reference. The primers utilised for PGC-1α mRNA were: forward 5’-
GCACTGACAGATGGAGACGTGAC-3’, and reverse 5’-
TCATTGTAGCTGAGCTGAGTGTTGG-3’, with produce size of 149 bp (accession number NM_031347.1). The parameters utilised in the PCR were: denaturation at 95°C for 5 min, followed by cycles of denaturation at 95°C for 15 s and annealing at 60°C for 15 s, for 40 circles. The melting curve analysis temperature was between 65°C to 95°C. The $2^{-ΔΔCT}$ method was used to determine the relative changes in mRNA (Livak & Schmittgen, 2001). In this study, the gene expression was presented as the fold change relative to the control group.

The expression of the protein was analysed using Western blotting technique. 100 mg of muscle samples were homogenised in 700 µL NP-40 lysing buffer. Due to small size of muscle samples available, samples from two subjects were mixed in homogenization, therefore three samples were analysed for each group. After centrifugation at 12,000 g for 20 min, the supernatant was extracted and added with 4X sample loading buffer (3:1), at 98°C for 5 min, and stored at -80°C before analysis. Protein extracts (0.1 mg) were resolved by SDS-PAGE. Electrophoresis was run with 5% stacking gel at 50 V for 50 min and 15% separating gel at 100 V for 90 min. The proteins were transferred to PVDF membranes (Millipore, MA) at constant current of 300 mA for 2 hours. The PVDF membranes were placed in culture and blocked by milk, shaking at room temperature for 1 h. Then the membrane with targeted band was incubated in 5%BSA buffer with the primary antibody (PGC-1α antibody #4295, CST Co., dilution 1:2000) at 4°C with gentle shaking overnight. The membrane was washed in TBST three times, each for 5 min, then incubated with the secondary antibody (Goat Anti-Rabbit HRP IgG, ZB-2301, ZSGB, China, dilution 1:10000) at room temperature with gentle shaking for 1 h. After three 5 min washes in TBST, the
samples were then incubated with luminescent substrate (Millipore, MA). Autoradiographs of the membranes were taken for visualisation of protein bands. The bands were quantified using Quantity ONE software (BIO-RAD), with GAPDH as the internal control.

One-way ANOVA (IBM SPSS version 20) was used to compare the mean values of the groups. The LSD was used for post-hoc between-groups comparisons, and alpha level was set at 0.05 for statistically significant differences. All the experimental procedures obtained approval by the University’s Animal Care and Ethics Committee.

Results

The first training session caused a significant increase of PGC-1α mRNA expression by 16.8 folds. During the subsequent training days, PGC-1α mRNA demonstrated a trend of increase in response to alternate-day training, and a trend of decrease in response to consecutive-day training (Figure 1), although the between-session differences did not reach statistical significance within each training protocol. A significant difference was found after three training sessions between the two training protocols (A3 > C4 and C7, A4 > C2-C7. Figure 1). The mRNAs of all training days were higher than the control ($P < 0.05$), except C7 which was lower than CA1, C2, and A2-A4 and no difference to CA0, C3 and C4.

The PGC-1α protein showed a trend of increase with both training protocols (74-77% increase at the end), however there were no statistically significant differences found between training sessions or between protocols (Figure 2).
Discussion

The major finding of this study was that, after a significant increase (16.8%) in response to the first training session, the PGC-1α mRNA demonstrated a trend of increase with further training sessions on alternate days (25.8% after four training sessions), and a trend of decrease relative to the first training session in consecutive training days, while it was still higher than the baseline level on day 4 (10.1%, \( p < 0.05 \)). Although the between-session differences did not show a statistically significant accumulation after the first session, the mRNA change in alternate-day training did become significantly higher than that of consecutive-day training after three training sessions.

Perry et al. (2010) reported that a 1 hour high-intensity interval cycling exercise induced a significant increase (11 folds above baseline) in PGC-1α mRNA expression in human muscle at 4 hour post exercise, and this transient change returned to baseline within 24 hours. With repeated training sessions (7 sessions) each separated by 1-2 days of recovery, the mRNA expression at 4 hour post increased after the first three training sessions, then gradually decreased with further training sessions (4 folds above baseline after 7 sessions), indicating no cumulative effect. Egan et al. (2013) examined gene expression changes in human muscle during two weeks of daily 60 min cycling exercise at 80%VO\(_{2}\)peak, with muscle biopsy samples taken on day 1, 3, 7, 10 and 14, at 16 h after the last training session. The authors reported that the expression of PGC-1α mRNA increased above the pre-training level at day 3 (48%), and declined thereafter but remained 25% higher than baseline on day 14. Because the samples were taken 16 hours after training, the elevated levels of mRNA could be regarded as a cumulative effect of training. The present study demonstrated a trend of continuous increase in acute response of PGC-1α mRNA in rat muscle, 1 hour post, with alternate-day training,
but a trend of decrease in consecutive-day training. Whether longer training period would result in significant cumulative effects require further study.

Interpretation of the mRNA changes must consider the sampling time post exercise, however the evidence in the current literature is equivocal. For instance, Pilegaard et al. (2003) obtained human muscle biopsy samples at 0, 2, 6 and 24 h post exercise and reported that the peak value of PGC-1α mRNA was found at 2 h post (Pilegaard et al. 2003). In another study, Pilegaard et al. (2005) analysed muscle biopsy samples obtained at 2, 5, 8 and 24 h after 75 min cycling at 75% VO$_2$max, and reported a significant increase of PGC-1α mRNA that peaked at 5 h (about 5 folds) and the mRNA returned to baseline level 24 h post exercise (Pilegaard et al. 2005). Norrbom et al. (2004) investigated the effects of 45 min knee extension exercise, and sampled at 0, 30 min, 2 h and 6 h post exercise (Norrbom et al. 2004). The PGC-1α mRNA showed no significant change at 0 and 30 min, but increased significantly at 2 h. The present study sampled at 1 h post exercise and showed significant increases in the PGC-1α mRNA expression. With reference to the above-mentioned reports, it could be speculated that a rapid change occurred in mRNA expression between 30 min to 60 min of recovery, although it is unknown whether the peak value was reached at 60 min and when it returned to baseline in rat muscle.

From these findings it appears that the transient changes of PGC-1α mRNA expression may not always show a cumulative effect with increased number of training sessions, and different patterns of change can be seen in various training protocols. It is known that the changes in mRNA expression do not affect the expression of proteins in a linear fashion (Egan et al. 2013; Hawley 2009; Perry et al. 2010). Perry et al. (2010) and Egan et al. (2013) both
reported a significant increase in PGC-1α protein expression after one training session (24 hour and 16 hours post, respectively), and a cumulative effect after further training sessions. In the current study, the PGC-1α protein showed a trend of increase with both training protocols, however the changes did not reach statistical significance, possibly due to the small samples size, large between-subject variations, and different sampling time.

Greater insight into PGC-1α expression may be provided from future studies with longer training period and on the expression of relevant downstream proteins in the signaling pathways of mitochondrial biogenesis. The outcomes of the research could be useful in optimizing training, by identifying molecular mechanisms of the adaptations to training programs with different exercise and recovery intervals.

Acknowledgements

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References


Table 1. Research design and protocols.

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<th>5</th>
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N = 6 in each group.

CA0: A common control group, no exercise, free access to water and food.

CA1: A common one day training group.

C2, C3, C4: Training on consecutive 2, 3 or 4 days.

C7: Training for 4 consecutive days and recovery for 3 days.

A2, A3, A4: A training day followed by a recovery day, for 2, 3, or 4 training days.

S: Euthanized for sampling.

T: Training for 90 minutes on a motor-driven treadmill, at slope of 10° and velocity of 19.3 m/min, stimulated by using a soft hair brush. This exercise intensity was at approximately ~76% $VO_2$ max for SD rats (Bedford et al. 1979).
Figure captions

Figure 1. PGC-1α mRNA responses to the two training protocols: A. training on alternate days in one week, and B. training on consecutive four days followed by 3 days of rest. *: \( P < 0.05 \), compared with CA0; §: \( P < 0.05 \), A4 > C2, C3, C4 and C7; ø: \( P < 0.05 \), A3 > C4 and C7; #: \( P < 0.05 \), C7 < CA1, C2, A2, A3 and A4.

Figure 2. PGC-1α protein response to the two training protocols: training on alternate days in one week (empty bars), and training on consecutive four days followed by three days of rest (filled bars). There were no significant differences found between the two training protocols or between training days. CA0 and CA1 were common control and 1 day training groups for the two protocols.
A. Training on alternate four days

B. Training on consecutive four days

253x309mm (300 x 300 DPI)