Effectiveness of sub-maximal intermittent exercise on muscle glycogen depletion, PGC-1α and PDK-4 gene expression

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Received 25 September 2012; revised 25 October 2012; accepted 7 November 2012

ABSTRACT

Several metabolic gene expressions are regulated in concert with muscle glycogen status. We hypothesized that intermittent exercise performed at high but sub-maximal intensities with long recovery periods would induce a low glycogen state that would stimulate peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1-α) and pyruvate dehydrogenase kinase-4 (PDK-4) gene expression in muscle. Nine young human subjects performed two intermittent exercise sessions. One session consisted of 60 s cycling bouts at VO2max (IE 100%), and the other session consisted of 75 s cycling bouts at 80% VO2max (IE 80%). Twelve bouts of exercise were completed in both sessions with a 4 min rest between each bout. Muscle specimens were obtained at pre-exercise and immediately, 1.5 h and 3 h post-exercise. Muscle glycogen was significantly decreased after both sessions (IE 100%, 94.1 ± 5.8 to 38.7 ± 5.5 mmol/kg w.w.; IE 80%, 94.6 ± 9.1 to 53.3 ± 4.8 mmol/kg w.w.; both P < 0.05 vs pre-exercise). Muscle glycogen depletion was greater in IE 100% than in IE 80% (P < 0.05). PGC-1α and PDK-4 mRNA expression were significantly increased after exercise in both sessions (IE 100%, PGC-1α: ~3.7 and ~2.9-fold, respectively; PDK-4: ~11.1 and ~3.5-fold, respectively; all P < 0.05). Maximal PDK-4 mRNA expression after exercise was significantly greater in IE 100% than in IE 80% (P < 0.05). In conclusion, high but sub-maximal intermittent exercise decreased muscle glycogen and stimulated PGC-1α and PDK-4 mRNA expression, suggesting that increasing exercise intensity contributes to muscle glycogen depletion and PDK-4 mRNA expression in human skeletal muscle.

Keywords: Intermittent Exercise; Muscle Glycogen; Peroxisome Proliferator-Activated Receptor-γ Coactivator-1α; Pyruvate Dehydrogenase Kinase-4

1. INTRODUCTION

Exercise training-induced increase in mitochondrial function and fat-oxidative capacity are result from the cumulative effects of transient increases in mRNA expression after successive exercise sessions [1]. A key genes of mitochondrial function and fat-oxidative capacity are peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and pyruvate dehydrogenase kinase-4 (PDK-4). PGC-1α expression was reported to be increased by an acute bout of exercise [2,3], and promoted expression of several mitochondria biogenesis-related genes (e.g. nuclear respiratory factors, transcription factor A) [4-6]. PDK-4 is highly expressed in skeletal muscle [7]. PDK-4 inactivates pyruvate dehydrogenase (PDH) by phosphorylating the E1 subunit. Inactivation of PDH impairs the conversion of pyruvate to acetyl-CoA, consequently switching the energy substrate from glucose to lipid [8]. PGC-1α and PDK-4 expression may be regulated in concert with muscle glycogen status. It was suggested that 5’AMP-activated protein kinase (AMPK), one of regulator of exercise induced PGC-1α expression, contains a glycogen binding domain [9-11], and phosphorylated levels of AMPK are increased with decreasing muscle glycogen [12-14]. PDK-4 transcriptional activation was also shown to be enhanced by a low muscle glycogen state via change in intracellular distribution of glycogen regulatory enzymes such as protein phosphatase 1 and glycogen synthase kinase 3 [15].

It was previously reported that the efficiency of muscle glycogen decreases with increasing exercise intensity [16]. However, high-intensity exercise is difficult to perform continuously, and the rate of glycogen oxidation
decreases with exercise duration [17]. Therefore, short-
term high-intensity Intermittent Exercise (IE) might be
useful for decreasing muscle glycogen. McCartney et al.
examined the metabolic change during four bouts of 30 s
of “all out” exercise, and reported that muscle glycogen
decreased dramatically after the first and second exercise
bouts, while glycogenolysis was inhibited during the
third and fourth exercise bouts [18]. An inhibition of gly-
cogenolysis enzyme (phosphofructokinase and phos-
sphorylase) activity by the decrease in muscle pH may
explain the inhibition of glycogenolysis by intense ex-
ercise [19,20]. Restoration of muscle pH is known to
occur progressively after exercise [21]. We previously
studied the effects of consecutive maximal exercise with
a long-term recovery period on muscle glycogen meta-
bolism, and suggesting that a sufficient recovery between
intense exercise has a favorable effect on muscle gly-
cogen decrease [22]. Nevertheless, a limitation of maxi-
mal IE is that it is extremely strenuous and difficult to
complete. Recently, Little et al. proposed a practical mo-
del of IE that consists of 8 - 12 one-minute bouts at
~100% of peak power output separated by a 75 s re-
covery [23]. In that study, IE stimulated mitochondrial
biogenesis-related gene expression. However, the levels
of muscle glycogen levels with exercise were not re-
ported.

In the present study, we hypothesized that high but
sub-maximal IE with a long recovery period between
exercise bouts would result in decreased muscle glyco-
gen and stimulation of PGC-1α and PDK-4 expression
without a heavy physical demand. To examine this hy-
pothesis, we compared the effects of two different IE
intensities (80% and 100% VO2max) with a 4 min re-
covery period on physiological responses, muscle gly-
cogen levels, and PGC-1α and PDK-4 gene expression.

2. MATERIAL AND METHODS

2.1. Subjects

Nine healthy men participated in this study (22 ± 1 year,
173.2 ± 3.4 cm, 64.1 ± 4.4 kg). No subjects were spe-
cifically engaged in training. The subjects were fully
informed of any risks and discomfort associated with the
IE sessions and muscle biopsy procedures before giving
their consent to participate. The study procedure was
approved by the ethical committee of Fukuoka Univer-
sity.

2.2. Measurement of VO2max

Before the first session, subjects made several fami-
larization visits to the laboratory to become oriented
with the study procedures. During one of these visits,
subjects performed an incremental test to exhaustion on
an electronically braked cycle ergometer (Rechor; Lode,
Groningen, The Netherlands) to determine VO2max
using an on-line gas collection system (ARCO 2000;
ARCO System, Chiba, Japan). The value used for VO2
max corresponded with the highest value achieved over a
30 s collection period. All subjects fulfilled at least one
of the two following VO2max criteria [24,25].

1) A plateau in VO2 where the increase in VO2 was
less than <150 ml/min with increasing workload.
2) Peak blood lactate concentration ≥8 mmol/L.

2.3. High-Intensity Intermittent Exercise

All subjects participated in two high-intensity IE sessions
conducted in random order at least 5 days apart. One of
the IE sessions consisted of 60 s cycling bouts at VO2max
(IE100%), and the other session consisted of 75 s cycling
bouts at 80% VO2max (IE80%). In both sessions, 12 bouts
of exercise were completed with a 4 min rest between
each bout, and the total work performed in each bout was
the same. The subjects were instructed to maintain their
normal diet throughout the duration of the research and
to refrain from alcohol consumption and strenuous exer-
cise for 24 h prior to each session (Figure 1).

On the day of each exercise session, the subjects
arrived at the laboratory between 6:30 am - 9:30 am after
an overnight fast. A catheter was inserted into the an-
tecubital vein, and a resting blood sample was taken at
least 15 min after the catheter insertion to minimize stress
reactions. Thereafter, resting muscle biopsies were obtained
using the fine-needle biopsy procedure. After a light
stretch, each subject performed the IE protocol at a stable
speed of approximately 60 rpm. Each subject was allow-
ed water, but no food, pre- and post-exercise.

During exercise, HR was collected every millisecond
using an electrocardiogram system (CARDIMAX FX-
3301; Fukuda Denshi Co., Tokyo, Japan). Outputs from
the electrocardiogram were collected on a personal

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computer using waveform processing software (Chart 5; AD Instruments Japan Inc., Nagoya, Japan) via an AD conversion board (PowerLab 8 sp; AD Instruments, Japan Inc.). All HR data were averaged every 1 s. Immediately after each exercise bout, ratings of perceived exertion (RPE) were obtained. To determine blood lactate concentration, 20 μL of blood was collected from the ear lobe. Blood samples were drawn from the antecubital vein after every three bouts. Further muscle biopsies were taken immediately, 1.5 h and 3 h post-completion of each session.

2.4. Blood Analysis

Blood samples were centrifuged at 3000× g at 4˚C for 15 min. Plasma and serum were stored at −30˚C for later analysis. Blood analysis included glucose (Hexokinase ultraviolet assay kit; Shino-Test, Tokyo, Japan), insulin (Chemiluminescent enzyme immunoassay kit; Fujirebio, Tokyo, Japan), triglyceride (Enzymatic kit; Sekisui Medical, Tokyo, Japan), free fatty acids (Enzymatic kit; Eiken Chemical, Tokyo, Japan), and catecholamine (High performance liquid chromatography method). All blood analyses were conducted by an independent company (SRL Inc., Tokyo, Japan).

2.5. Muscle Biopsy Procedures

Muscle samples were taken using a previously described method [26]. Muscle biopsy tissue specimens (approximately 10 mg) were obtained from middle of the vastus lateralis of the dominant leg using a Bard MAGNUM biopsy instrument (C. R. Bard; Covington, GA, USA) with a 16 g disposable needle. Lidocaine tape agent (Penles; Nitto Denko Corp., Tokyo, Japan) was applied to the biopsy site at > 2 h before biopsy. To facilitate the insertion of the biopsy needle, a hole was opened in the skin with an 18 g injection needle immediately before the biopsy. After insertion, the trigger was pushed with the fine needle perpendicular to the hole of the skin. Blood was removed immediately from the biopsy tissue specimen using gauze, and was frozen with liquid nitrogen and stored at −80˚C until analysis.

2.6. Gene Expression Analysis

Total RNA was extracted from approximately 5 mg of freeze-dried muscle. All samples were homogenized using a POLYTRON homogenizer. mRNA was extracted using the Maxwell® LEV System (Promega, Tokyo, Japan). Real-time PCR (Light Cycler; Roche Diagnostics, Tokyo, Japan) was used for qualitative analysis of gene expression. The specific genes analyzed and the primers used are presented in Table 1. Real-time PCR was performed with a SYBR Green I (Light Cycler Fast Start DNA Master SYBR Green I; Roche Diagnostics) reagent.

The specificity of the PCR product was identified using a melting curve analysis that determined the temperature of denaturation of the PCR product through a heating procedure. The melting curve patterns were compared by visual inspection. 36B4 was used as a housekeeping gene to normalize target mRNA. The average CT values of the unknown samples were converted to relative expression data using an appropriate standard curve.

2.7. Muscle Glycogen Measurement

Muscle glycogen content was determined using previously reported procedures [27]. Muscle (2 - 5 mg) was dissolved in 30% KOH and 5% Na2SO4 at 70˚C for 15 min. Glycogen was then precipitated by mixing with an absolute alcohol, and was stored at −20˚C overnight. The precipitates were collected by centrifugation at 13,000 × g for 5 min. Glycogen was hydrolyzed in 6 N H2SO4 at 100˚C for 45 min and cooled. Samples were neutralized with 1 N NaOH, and glucose was measured using the glucose (HK) reagent (Sigma, Saint Louis, MO, USA). The intra-assay coefficient of variation (CV) was 6.9% - 7.2%.

2.8. Statistical Analysis

All data are expressed as mean ± SEM. PDK-4 and PGC-1α mRNA abundance were normalized to 36B4 mRNA abundance, and post-exercise sample data were expressed relative to the corresponding pre sample that was set to one. Two-way ANOVAs with repeated measures were used to evaluate the interaction of time and condition in IE100% and IE80%, followed by Tukey-Kramer’s post-hoc test and Student’s paired t-test to locate the differences when significant main effects were

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene bank accession number</th>
<th>Forward primer (5’ - 3’)</th>
<th>Reverse primer (5’ - 3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α</td>
<td>AF106698</td>
<td>ACCGTATACCTGTGATGC</td>
<td>CCAGAGAGTCACTTGTC</td>
<td>186</td>
</tr>
<tr>
<td>PDK-4</td>
<td>NM_002612</td>
<td>GCCCCGTCGCCGCTGTC</td>
<td>TCTGGTCACTCTGTTTCTCAT</td>
<td>240</td>
</tr>
<tr>
<td>36B4</td>
<td>M177885</td>
<td>AGTTGTCCAGATAACGACATCTAC</td>
<td>TCGTTTGATACCGTGTATAGA</td>
<td>140</td>
</tr>
</tbody>
</table>
found. Differences were considered significant at \( P < 0.05 \). Statistical calculations were performed using PASW Statistic 18 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Physiological Responses and Blood Metabolism

HR, RPE, and blood lactate concentration during exercise are shown in Table 2. Exercise-induced increases in HR and RPE demonstrated a significant main effect for exercise session protocol, with no significant interactions. The exercise-induced increase in blood lactate concentration was significantly greater in IE100% compared with IE80% (\( P < 0.05 \)). IE100% and IE80% increased blood triglyceride, adrenaline, and noradrenaline, and decreased blood Free Fatty Acids (FFA) concentration (main effect for time, \( P < 0.01 \)). For all blood biochemistry measurements, there were no significant interactions (Table 3).

3.2. Muscle Glycogen

At rest, muscle glycogen contents were similar between IE100% and IE80%, and were decreased significantly after exercise in both sessions (IE100%, 94.1 ± 5.8 to 38.7 ± 5.5 mmol/kg w.w; IE80%, 94.6 ± 9.1 to 53.3 ± 4.8 mmol/kg w.w; \( P < 0.05 \) vs pre-exercise for both). The rate of muscle glycogen depletion was significantly greater in IE100% compared with IE80% (58.5 ± 5.3% vs 43.1 ± 2.7%; \( P < 0.05 \)).

3.3. Expression of PGC-1α and PDK-4 Genes

The IE100% and IE80% significantly increased PGC-1α mRNA abundance ~3.7-fold and ~2.9-fold, respectively, throughout the 3 h recovery period (main effect for time, \( P < 0.01 \)).

Table 2. HR, RPE, and blood lactate concentrations during exercise.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>HR (bpm)</th>
<th>Rest</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE100%</td>
<td>74 ± 4</td>
<td>122 ± 5</td>
<td>130 ± 4</td>
<td>134 ± 4</td>
<td>138 ± 4</td>
<td>140 ± 4</td>
<td>142 ± 4</td>
<td>144 ± 3</td>
<td>145 ± 4</td>
<td>146 ± 4</td>
<td>146 ± 4</td>
<td>148 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE80%</td>
<td>73 ± 3</td>
<td>118 ± 5</td>
<td>125 ± 5</td>
<td>129 ± 4</td>
<td>133 ± 4</td>
<td>135 ± 4</td>
<td>136 ± 4</td>
<td>137 ± 4</td>
<td>136 ± 4</td>
<td>138 ± 4</td>
<td>139 ± 4</td>
<td>140 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR†#</td>
<td>RPE†#</td>
<td>IE100%</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE80%</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA#†$</td>
<td>IE100%</td>
<td>1.2 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>6.1 ± 0.2</td>
<td>6.6 ± 0.2</td>
<td>7.0 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>7.1 ± 0.3</td>
<td>7.2 ± 0.3</td>
<td>7.3 ± 0.3</td>
<td>7.3 ± 0.3</td>
<td>7.2 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE80%</td>
<td>1.0 ± 0.1</td>
<td>3.5 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>4.1 ± 0.3</td>
<td>4.1 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 9). †Significant main effect for time. #Significant main effect for condition. $Significant interaction between time and condition. *Significant difference compared to rest value in same experimental protocol.

Table 3. Blood glucose, insulin, FFA, triglyceride, adrenaline, and noradrenaline concentrations during exercise.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Glucose (mg/dL)</th>
<th>Rest</th>
<th>After 3 bouts</th>
<th>After 6 bouts</th>
<th>After 9 bouts</th>
<th>After 12 bouts</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE100%</td>
<td>93.4 ± 2.0</td>
<td>92.6 ± 1.8</td>
<td>91.4 ± 1.8</td>
<td>92.2 ± 2.2</td>
<td>92.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>IE80%</td>
<td>91.6 ± 1.4</td>
<td>92.3 ± 1.2</td>
<td>90.3 ± 1.3</td>
<td>89.3 ± 1.3</td>
<td>87.8 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Insulin (μIU/mL)</td>
<td>IE100%</td>
<td>6.18 ± 0.89</td>
<td>6.93 ± 0.72</td>
<td>6.64 ± 0.72</td>
<td>6.95 ± 1.01</td>
<td>6.82 ± 0.88</td>
</tr>
<tr>
<td>IE80%</td>
<td>5.62 ± 0.82</td>
<td>5.64 ± 0.33</td>
<td>5.80 ± 0.56</td>
<td>5.44 ± 0.55</td>
<td>4.98 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>FFA (μEq/L)</td>
<td>IE100%</td>
<td>602 ± 99</td>
<td>356 ± 52*</td>
<td>279 ± 38*</td>
<td>244 ± 37*</td>
<td>245 ± 50*</td>
</tr>
<tr>
<td>IE80%</td>
<td>596 ± 77</td>
<td>377 ± 51*</td>
<td>287 ± 35*</td>
<td>270 ± 33*</td>
<td>280 ± 38*</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>IE100%</td>
<td>64.2 ± 10.6</td>
<td>70.9 ± 12.1</td>
<td>69.3 ± 12.2</td>
<td>66.4 ± 12.1</td>
<td>63.7 ± 12.0</td>
</tr>
<tr>
<td>IE80%</td>
<td>75.9 ± 11.8</td>
<td>72.1 ± 10.2</td>
<td>68.1 ± 9.6*</td>
<td>65.4 ± 9.6*</td>
<td>63.6 ± 9.0*</td>
<td></td>
</tr>
<tr>
<td>Adrenaline (pg/mL)</td>
<td>IE100%</td>
<td>54.2 ± 15.6</td>
<td>84.4 ± 14.9*</td>
<td>84.9 ± 9.9*</td>
<td>98.7 ± 14.9*</td>
<td>109.8 ± 11.8*</td>
</tr>
<tr>
<td>IE80%</td>
<td>44.9 ± 12.9</td>
<td>74.3 ± 9.9*</td>
<td>95.0 ± 10.1*</td>
<td>88.9 ± 9.6*</td>
<td>105.6 ± 11.9*</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (pg/mL)</td>
<td>IE100%</td>
<td>280 ± 53</td>
<td>700 ± 70*</td>
<td>698 ± 70*</td>
<td>823 ± 101*</td>
<td>824 ± 98*</td>
</tr>
<tr>
<td>IE80%</td>
<td>338 ± 67</td>
<td>668 ± 104*</td>
<td>675 ± 107*</td>
<td>689 ± 100*</td>
<td>692 ± 101*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 9). †Significant main effect for time. #Significant main effect for condition. *Significant difference compared with rest value in same experimental protocol.
was greater for IE\textsubscript{100\%} than IE\textsubscript{80\%}. These data are supported by a previous study showing that the efficiency of the decrease in muscle glycogen is enhanced with increasing exercise intensity [16]. Considering the change of muscle glycogen content in the present study, it is possible that the decrease in muscle pH during IE\textsubscript{100\%} and IE\textsubscript{80\%} was not enough to inhibit glycogenolysis. Unfortunately, we did not measure muscle pH in the present study, while blood lactate concentrations during IE\textsubscript{100\%} and IE\textsubscript{80\%} were nearly 7 mmol/L and 4 mmol/L, respectively. These values were less than half of the blood lactate concentration reported in a previous study demonstrating glycogenolysis inhibition during IE [18]. Thus, the 4 min rest period during sub-maximal IE may have been sufficient to promote a decrease in glycogen.

Similar to previous reports [28,29], we found that PGC-1\textalpha and PDK-4 gene expression were significantly increased in the 3 h post-exercise in IE\textsubscript{100\%} and IE\textsubscript{80\%}. PGC-1\textalpha expression is mainly regulated by AMPK [9,10], p38 mitogen-activated protein kinase [30], Ca\textsuperscript{2+}-calmodulin dependent kinase II [31], and catecholamine stimulation [32]. Unfortunately, we were unable to measure these kinases, although it was reported that muscle glycogen availability relates negatively to AMPK activity [12-14]. Therefore, we speculate that the increase in AMPK activity related to the decrease in muscle glycogen and blood catecholamine concentrations may have stimulated PGC-1\textalpha mRNA expression in IE\textsubscript{100\%} and IE\textsubscript{80\%}. On the other hand, we found no relationship between muscle glycogen content and PGC-1\textalpha mRNA expression. Mathai et al. previously reported a strong association between changes in muscle glycogen content and PGC-1\textalpha mRNA expression. Mathai et al. previously reported a strong association between changes in muscle glycogen content and PGC-1\textalpha protein content in a wide range of conditions, including muscle glycogen depleted and supercompensated state [33]. However, this relationship seems weak when examining only the glycogen depleted state. Therefore, the difference between the sessions in terms of muscle glycogen depletion is insufficient to explain the relationship between muscle glycogen content and PGC-1\textalpha expression. In accordance with previous findings showing that PDK-4 gene expression is stimulated in a low muscle glycogen state [15,34], we found that muscle glycogen content was approximately 20% lower and the maximum value of PDK-4 mRNA throughout the 3 h recovery was 3.1-fold higher after IE\textsubscript{100\%} compared with IE\textsubscript{80\%}. Therefore, we speculate that the low muscle glycogen state after IE\textsubscript{100\%} and IE\textsubscript{80\%} contributed to the increased in PDK-4 mRNA expression with decreasing muscle glycogen levels. Elevated blood FFA and reduced blood insulin were also reported to contribute to PDK-4 gene expression [34,35]. However, we found that blood FFA concentration decreased, while blood insulin concentration was not changed, during both sessions, suggesting that changes in these blood metabolites and
hormones were not associated with PDK-4 mRNA expression. Spriet et al. reported that PDK-4 mRNA expression after both sessions was increased with advancing fasting duration [36]. In the present study, all subjects started exercise in a 10 h fasted state and were not allowed water until 3 h post exercise. Therefore, it is possible that exercise-induced PDK-4 expression after both sessions may be affected by the fasting state. The PGC-1α and PDK-4 results in the present study are partly supported by findings of Cluberton et al. where glucose ingestion before exercise attenuated the exercise-induced increase in PDK-4 mRNA expression, whereas the exercise-induced increase in PGC-1α mRNA expression was not affected by the change in glucose-availability [37]. Based on these results, we speculate that PDK-4 expression has a strong negative relationship with carbohydrate (blood glucose and muscle glycogen), whereas PGC-1α expression has a weak negative relationship with carbohydrate.

In the present study, importantly, we also determined the physical demands of the IE protocols that use a long recovery period. In IE100% and IE80%, the peak HR during exercise corresponded to 79.5% and 71.3% of HR reserve, respectively, which were relatively low compared with the value permitted by the American College of Sports Medicine’s Exercise Guidelines for health in healthy people [23]. In addition, the RPE during the exercise session did not increase to extremely high levels (e.g. IE100%, 12 - 16; IE80%, 11 - 14). The IE100% and IE80% consisted of a combination of ~75 s exercise bouts each separated by a 4 min recovery. These data suggest that this exercise pattern is tolerable and does not place strenuous physical demands on individuals. In addition to these results, a recent report indicated that PGC-1α can coactivate genes associated with muscle plasticity and mitochondrial biogenesis as well as the suppression of inflammation [38]. Therefore, IE100% and IE80% have the potential to be effective exercise methods for both athletes and non-athletes.

5. CONCLUSION

We found that IE protocols with 4 min recovery periods between each 1 min exercise bout (IE100% and IE80%) markedly decreased muscle glycogen content and increased PGC-1α and PDK-4 mRNA expression. In addition, increasing exercise intensity of IE elevated muscle glycogen depletion and PDK-4 expression.

6. ACKNOWLEDGEMENTS

This study was performed with the support of the Fukuoka University Institute for Physical Activity, a Technology Scientific Research Budget Basic Research Grant (A19200049 Strategic Research Infrastructure) from the Japanese Government’s Ministry of Education, Culture, Sports, Science and Technology (MEXT), and MEXT-Supported Program for Strategic Research Foundation at Private Universities, 2008-2012. We would like to thank all subjects for their time and effort in this study.

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