# Sprint Interval Training in Hypoxia Stimulates Glycolytic Enzyme Activity

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**ABSTRACT**

PUYPE, J., K. VAN PROEYEN, J.-M. RAYMACKERS, L. DELDICQUE, AND P. HESPEL. Sprint Interval Training in Hypoxia Stimulates Glycolytic Enzyme Activity. *Med. Sci. Sports Exerc.*, Vol. 45, No. 11, pp. 2166–2174, 2013. **Purpose**: In this study, we compared the effect of sprint interval training (SIT) in normoxia versus hypoxia on muscle glycolytic and oxidative capacity, monocarboxylate transporter content, and endurance exercise performance. **Methods**: Healthy male volunteers (18–30 yr) performed 6 wk of SIT on a cycling ergometer (30-s sprints vs 4.5-min rest intervals; 3 $d \cdot wk^{-1}$) in either normobaric hypoxia (HYP, $FiO_2 = 14.4\%$, $n = 10$) or normoxia (NOR, $FiO_2 = 20.9\%$, $n = 9$). The control group did not train (CON, $n = 10$). Training load was increased from four sprints per session in week 1 to nine sprints in week 6. Before and after SIT, subjects performed a maximal incremental exercise test plus a 10-min simulated time trial on a cycle ergometer in both normoxia ($MAX_{nor}$ and $TT_{nor}$) and hypoxia ($MAX_{hyp}$ and $TT_{hyp}$). A needle biopsy was taken from musculus vastus lateralis at rest 5–6 d after the last exercise session. **Results**: SIT increased muscle phosphofructokinase activity more in HYP (+59%, $P < 0.05$) than that in NOR (+17%), whereas citrate synthase activity was similar between groups. Compared with the pretest, power outputs corresponding to 4 mmol blood lactate in HYP during $MAX_{nor}$ (+7%) and $MAX_{hyp}$ (+9%) were slightly increased ($P < 0.05$), whereas values were constant in NOR. $\dot{V}O_{2max}$ in $MAX_{nor}$ and TT performance in $TT_{nor}$ and $TT_{hyp}$ were increased by ~6%–8% ($P < 0.05$) in either group. The training elevated monocarboxylate transporter 1 protein content by ~70% ($P < 0.05$). In CON, all measurements were constant throughout the study. **Conclusion**: SIT in hypoxia up-regulated muscle phosphofructokinase activity and the anaerobic threshold more than SIT in normoxia but did not enhance endurance exercise performance.
**Key Words:** HIGH-INTENSITY INTERVAL TRAINING, ANAEROBIC THRESHOLD, PHOSPHOFRUCTOKINASE, MUSCLE LACTATE TRANSPORTER, ENDURANCE PERFORMANCE

Altitude training has been used for more than 50 yr as a popular strategy in athletic populations to improve endurance exercise performance at sea level. For the last 15–20 yr, various new technologies have been developed to simulate altitude environments by the production of hypoxic air under normobaric conditions. This has stimulated the use of the so-called "live high–train low" procedures in the preparation of endurance competitions because athletes can comfortably embed "altitude" in their normal training environment at sea level. In addition, some of the commercial hypoxicators can produce hypoxic air at sufficiently high rates to cope with the high pulmonary ventilations elicited by high-intensity exercise. Hence, "living low–training high" also is becoming a popular ingredient of training in endurance athletes. The validity of such an approach has been demonstrated in a series of studies showing high-intensity endurance training in hypoxia to be more effective than similar training in normoxia, to induce skeletal muscle remodeling to facilitate oxidative energy turnover (22,40). Thus, training in hypoxia was found to activate hypoxia-inducible factor 1 (HIF-1), which was associated with increased mitochondrial volume density and capillarity, as well as the up-regulation of vascular endothelial growth factor and glycolytic enzyme mRNAs (35,40). Furthermore, some studies have indeed found increased $\dot{V}O_{2max}$ and maximal power outputs, but most prominently during exercise testing at altitude (23).

Apart from hypoxia training, sprint interval training (SIT), characterized by repeated sprints at supramaximal workloads interspersed by short recovery bouts, also is a popular strategy to enhance endurance exercise performance. It has been demonstrated that a few weeks of consistent SIT, involving 30-s "all-out" sprints interspersed by 4 min of active recovery episodes, can increase indices of muscular oxidative capacity (3,6–8,13,17), glycolytic energy turnover (17), $\dot{V}O_{2max}$ (3,7,17,21), and exercise performance (6,8,13,17,21), to the same degree or even more than much larger volumes of aerobic endurance training (7,13). Hence, small-volume

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SIT may be a time efficient strategy to stimulate muscle and performance adaptations equal to traditional high-volume endurance training.

To date, experiments involving hypoxic training have just looked at the potential of traditional constant-load "aerobic" exercise. However, on the basis of the aforementioned evidence, it is reasonable to speculate that SIT in hypoxia might be another pertinent exercise stimulus to boost endurance exercise performance either at sea level or at altitude. It is well established that aerobic performance capacity consistently drops at altitude (36), which impairs training adaptation due to reduction of overall training workload. Conversely, performance in short maximal exercise is not impaired by acute exposure to low or moderate altitude/hypoxia (38) because increased fraction of glycolytic ATP production compensates for the hypoxia-induced drop of oxidative energy input. Arterial oxygen content in fact is significantly reduced during SIT in hypoxia (30). Hence, a lower rate of oxygen delivery to muscles increases stress on glycolytic flux, which conceivably may stimulate the up-regulation of this energy pathway. Against this background, in this study, we compared the effects of a well-validated high-intensity interval training protocol (7) in normoxia versus hypoxia (14.5% $F_iO_2$, corresponding to ~3000 m altitude) on $\dot{V}O_{2max}$ and endurance exercise performance in both normoxia and hypoxia as well as on indices of muscle oxidative and glycolytic capacity.

## METHODS

### Subjects

Twenty-nine healthy male subjects, age 18–30 yr, volunteered to take part in the study, which was approved by the ethics committee of KU Leuven. Subjects gave their written, informed consent after they underwent a medical checkup and were informed in detail of all experimental procedures and possible risks associated with the experiments. Exclusion criteria for participation were smoking, exposure to hypoxia or intermittent sprint training during a period of 6 months before the study, and any health risk that could compromise the subject's safety during training and/or hypoxia exposure. All subjects were involved in regular sports and physical activity at a rate of three to five times per week. Subjects were instructed not to participate in any strenuous exercise sessions other than prescribed by the study protocol. They were also repeatedly instructed to maintain constant dietary and physical activity pattern throughout the study and to refrain from any exercise, except the activities of daily living, for 2 d before the experimental sessions (pretest and posttest). Furthermore, to minimize diet-induced variability in muscle metabolism during exercise, subjects were instructed to register their food intake in a diary for 3 d preceding the pretest and to replicate this diet for the posttest. In addition, during the pretest and posttest, subjects received a carbohydrate-rich meal (1050 kcal, 60% carbohydrates, 30% fat, 10% protein) between 3 and 4 h before the start of the exercise tests. During the study, one subject from the normoxia training group (see next section) was excluded because of nonadherence to the training protocol.

### Experimental Protocol

**Study design and experimental groups.** The subjects first performed a familiarization session to experience the procedures of the exercise tests for the pretest and the posttest. One week later, they participated in the pretest, which involved two identical exercise testing sessions, interspersed by a 2-d rest interval, including an incremental $\dot{V}O_{2max}$ test plus a 10-min simulated time trial. However, the first session was performed in normoxia ($F_iO_2$ ~20.9%), whereas the second was performed in hypoxia ($F_iO_2$ ~14.5%). In addition, 5–6 d after the last session, the subjects returned to the laboratory after an overnight fast for a resting muscle biopsy. Needle biopsies were taken from the right musculus vastus lateralis through an incision in the skin and under local anesthetic (2–3 mL lidocaine, subcutaneously). After the pretest, subjects were matched to obtain triplets with similar values for age, body weight, and power output at $\dot{V}O_{2max}$ (Table 1). The triplets were randomly assigned to three experimental groups. Two groups participated in a 6-wk supervised cycling training program involving SIT (see next section) in either hypoxia (HYP, $n$ = 10) or normoxia (NOR, $n$ = 9). A control group (CON, $n$ = 10) was not enrolled in the training program. After the 6-wk intervention period, all subjects participated in the posttest, which was identical with the pretest. Exercise tests in the pretest and the posttest were performed on the same day of the week and same time of the day. In the posttest, to allow for adequate recovery, the last SIT session was scheduled 3 d before the start of the posttest. Furthermore, because muscle biopsies were taken 5–6 d after the last exercise test session, biopsies in the posttest were taken 12–13 d after the last SIT session.

**Training intervention.** NOR performed all SIT sessions in normoxia ($F_iO_2$ ~20.9%), whereas HYP trained in a normobaric hypoxic facility (SportingEdge, Sherfield on Loddon, UK) maintained at 14.5% ambient $O_2$ content, which corresponds to ~3000 m of simulated altitude. Subjects were not blinded to the conditions because the perception of exaggerated hyperventilation during recovery from sprints in hypoxia conceivably would rapidly undo the blinding. Subjects participated in three supervised training sessions per week (Monday–Wednesday–Friday) for 6 wk. Each SIT session started with a 10-min warm-up at 100 W. Thereafter, subjects performed a series of 30-s sprints, separated by recovery cycling intervals of 4 min 30 s (50 W). Cadence during the sprints was fixed at 100–105 rpm by using the isokinetic mode of operation of the cycle ergometers (Avantronic Cyclus 2). In each session, subjects were asked to perform the first and the last sprint at maximal power. The other sprints were performed at ~80% of the

mean power output measured in the first sprint. The number of sprints per session was progressively increased from four in week 1 to nine in week 6. Immediately after the last sprint, the subjects started a 15-min cooling down at 100 W. Power output during the sprints was continuously measured (Avantronic Cyclus 2). Furthermore, 3 min after the first and the last sprint, a capillary blood sample (5 $\mu$L) was collected from an earlobe for determination of blood lactate concentration (Lactate Pro, Arkray, Japan). Pilot experiments in a similar population in our laboratory before the start of the study indicated that arterial oxygen saturation during SIT in 14.5% ambient $O_2$ content is consistently reduced compared with similar training in normoxia (Fig. 1).

**Exercise testing.** In the pretest and the posttest, the subjects reported to the laboratory in normoxia ($F_iO_2$ ~20.9%) to perform a maximal incremental exercise test ($MAX_{nor}$) followed by a 10-min simulated time trial ($TT_{nor}$) on an electromagnetically braked bicycle ergometer (Avantronic Cyclus 2). Three days later, these tests were repeated in hypoxia ($F_iO_2$ ~14.5%, $MAX_{hyp}$ and $TT_{hyp}$). The initial workload for the incremental $\dot{V}O_{2max}$ tests was set at 60 W and was increased by another 35 W every 3 min until voluntary exhaustion (drop of cadence <75 rpm). During the test, heart rate was continuously measured (Polar, Kempele, Finland), and a capillary blood sample (5 $\mu$L) for lactate assay (Lactate Pro) was taken from an earlobe during the final 30 s of each stage as well as 0 and 3 min postexercise. The exercise intensity (W) corresponding with a blood lactate level of 4 mmol·L$^{-1}$ (LT4) was extrapolated from the lactate curve and was used as an index of the anaerobic threshold. Furthermore, during the final 3–5 min of the test, expired gases were collected to determine $\dot{V}O_{2max}$ (Cortex® Metalyzer II, Leipzig, Germany). The subjects then actively recovered for 20 min by easy cycling at 60 W, after which the 10-min TT was started. Initial load was set at 90% of peak power output obtained from the $\dot{V}O_{2max}$ test, and subjects could adjust the workload at any time, aiming to develop as high as possible a mean power output during the 10 min. However, power output was hidden to the subjects for workload adjustments would be driven by rate of perceived exertion only. Heart rate during the time trial was continuously measured (Polar), and a capillary blood sample for determination of lactate concentration (Lactate Pro) was sampled after 3 min of passive recovery.

### Analysis of Muscle Samples

A part of the muscle samples was immediately frozen in liquid nitrogen. All muscle samples were stored at −80°C till later analysis. Muscle glycogen content was measured as glucose residues after acid hydrolysis using a standard enzymatic fluorometric assay (19). Maximal activities of citrate synthase (CS) and phosphofructokinase (PFK) were performed using enzymatic spectrophotometric assays as previously described (11). For Western blotting, approximately 30 mg of muscle was first homogenized (Polytron 2100, Kinematica, Newark, NJ) in a sucrose buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, 2 mM PMSF, pH 7.4) and centrifuged at 1000*g* for 5 min. This procedure removed heavy material, including a fraction of the mitochondria. The supernatant was spun at 190,000*g* for 90 min at 4°C. The new supernatant (cytosolic fraction) was stored at –80°C, whereas the new pellet (total muscle membrane fraction, including sarcolemmal and mitochondrial membrane fractions) was resuspended in Tris–sodium dodecyl sulfate (SDS) (10 mM Tris, 4% SDS, 1 mM EDTA, 2 mM PMSF, pH 7.4). Protein content was determined with a bovine serum albumin standard (DC protein assay; Bio-Rad, Herlev, Denmark). Fifteen micrograms of protein from each sample was combined with Laemmli sample buffer and subjected to SDS–polyacrylamide gel electrophoresis. After electrophoretic separation at 40 mA for 1 h, the proteins were transferred to a polyvinylidene fluoride membrane at 80 V for 2 h for Western blot analysis. Membranes were then incubated in 5% milk powder in tris buffered saline with Tween 20. Subsequently, membranes were incubated with the following antibodies overnight at 4°C: phosphoadenosine monophosphate–activated protein kinase alpha (P-AMPK$\alpha$Thr$^{172}$; Cell Signaling Technology, Danvers, MA), total adenosine monophosphate–activated protein kinase (total AMPK; Cell Signaling), eukaryotic elongation factor 2 (eEF2; Cell Signaling), monocarboxylate transporter 1 (MCT1; Millipore, Overijse, Belgium), and MCT4 (Millipore). Membranes were washed in TBS-T and incubated for 1 h at room temperature in a secondary antibody conjugated to horseradish peroxidase. After an additional three washes, chemiluminescence detection was carried out using Luminate™ Forte Substrate (Millipore). Membranes were scanned and quantified with Genetools and Genesnap softwares (Syngene, Cambridge, UK), respectively. Results were expressed relative to a standard sample that was run together with the samples and are reported relative to AMPK total for P-AMPK and eEF2 total for MCT1 and MCT4. Pretest values in CON were assigned the arbitrary value of 1.0, and all other samples were expressed relative to this value.

### Data Analysis and Statistical Analyses

The effects of the interventions were evaluated using a repeated-measures ANOVA. Two-way ANOVA was performed to examine the main effects of treatment and/or time. A planned contrast analysis was used for *post hoc* comparisons when appropriate. Contrast analysis was also used to evaluate specific preplanned comparisons. A probability level ($P$) ≤0.05 was considered statistically significant. All data are expressed as means ± SEM.

## RESULTS

**Sprint training parameters.** The number of sprints per session was progressively increased from four to nine throughout the 6-wk training period (see Table 2). Hence,

TABLE 1. Subject characteristics.

| | CON | NOR | HYP |
|---|---|---|---|
| Age (yr) | 27.9 ± 2.0 | 25.1 ± 1.9 | 23.8 ± 1.6 |
| Height (cm) | 179.9 ± 2.5 | 179.2 ± 2.2 | 179.3 ± 1.6 |
| Weight (kg) | 79.7 ± 3.0 | 74.4 ± 2.5 | 75.3 ± 2.5 |
| $\dot{V}O_{2max}$ (mL·min$^{-1}$·kg$^{-1}$) | 55.1 ± 2.5 | 53.3 ± 3.5 | 55.1 ± 1.7 |
| Peak power (W) | 322 ± 16 | 310 ± 14 | 305 ± 9 |

Values are presented as mean ± SEM for the control group (CON, $n = 10$) and the groups training in either normoxia (NOR, $n = 9$; $F_iO_2$, 20.9% ) or hypoxia (HYP, $n = 10$; $F_iO_2$, 14.5%).

from the start to the end of the training, the mean power outputs during the last sprint of each session did not significantly increase. However, the mean power outputs in the first sprints increased by ~5% ($P < 0.05$) in both training groups. Nonetheless, peak power outputs were stable from the pretest to the posttest in either group. Irrespective of the experimental condition, blood lactate concentration increased from ~7 to 8 mmol·L$^{-1}$ in the first sprint to ~13 to 14 mmol·L$^{-1}$ in the final sprint in both week 1 and week 6 ($P < 0.05$). Peak heart rates during the training were similar between the groups at all times.

**Incremental exercise tests.** All subjects performed a maximal incremental exercise test in both normoxia ($MAX_{nor}$, $F_iO_2$, 20.9%) and in hypoxia ($MAX_{hyp}$, $F_iO_2$, 14.5%; see Table 3). In the pretest, power output at LT4 as well as peak $\dot{V}O_2$, blood lactate, and time to exhaustion were similar between the groups. Power output at LT4 (~17%), peak $\dot{V}O_2$ (~15%), time to exhaustion (~15%), and peak heart rate (~3%) were significantly lower in $MAX_{hyp}$ than that in $MAX_{nor}$. In CON, most measurements were constant between the pretest and the posttest, except peak $\dot{V}O_2$ in $MAX_{hyp}$, which slightly dropped ($P < 0.05$). Both training groups augmented peak $\dot{V}O_2$ by ~6.5% ($P < 0.05$) in $MAX_{nor}$, but not in $MAX_{hyp}$. In $MAX_{nor}$, compared with the pretest, power output in the posttest at LT4 increased by ~7% in HYP ($P < 0.05$) but was unchanged in NOR (HYP vs NOR, $P = 0.09$). Similarly, the training intervention increased LT4 power output during $MAX_{hyp}$ by ~9% in HYP ($P < 0.05$), but not in NOR. Furthermore, from the pretest to the posttest, time to exhaustion in both NOR and HYP was improved by ~4% in $MAX_{nor}$ ($P < 0.05$) versus ~7% in $MAX_{hyp}$ ($P < 0.05$). Irrespective of the treatment, peak heart rate, blood lactate, and RER were similar between the pretest and the posttest in either $MAX_{nor}$ or $MAX_{hyp}$.

**Time trial performance.** Similar to the incremental exercise tests, the subjects performed the 10-min simulated time trial in both normoxia ($TT_{nor}$, $F_iO_2$, 20.9%) and in hypoxia ($TT_{hyp}$, $F_iO_2$, 14.5%; see Table 4). In the pretest, mean power output was not significantly different between the three groups, yet on average was ~15% lower in $TT_{hyp}$ than that in $TT_{nor}$. TT performance in CON was unchanged in the posttest. Irrespective of the experimental condition, the training period increased mean power output by ~6%–8% in both TTs ($P < 0.05$). In NOR, compared with the pretest, blood lactate concentration in the posttest was higher in $TT_{nor}$ and $TT_{hyp}$ ($P < 0.05$). Conversely, in HYP, blood lactate was higher in the posttest than that in the pretest in $TT_{hyp}$ ($P < 0.05$), but not in $TT_{nor}$. However, lactate values at the end of the TTs were not significantly different between NOR and HYP at any time.

**Muscle enzyme activity.** PFK and CS activity in resting muscle were used as indices of muscle glycolytic and oxidative capacity, respectively (see Figure 2). Pretest values for either enzyme were similar between the groups and in CON were constant between the pretest and the posttest. The training program markedly increased PFK activity in HYP (+59%, $P < 0.05$), but not in NOR. Thus, in the posttest, PFK activity was significantly higher in HYP than that in NOR ($P < 0.05$). Irrespective of the experimental condition, CS activity was constant from the pretest to the posttest.

**Muscle lactate transporters, AMPK, and glycogen content.** In the pretest, muscle MCT1 and MCT4 protein content and glycogen content were similar between the groups, and in CON values were constant between the pretest and the posttest (see Figure 2). The training increased muscle MCT1 protein content by ~70% in both NOR and HYP ($P < 0.05$), whereas MCT4 contents were unchanged. AMPK total protein content and phosphorylation status were constant between the pretest and the posttest, and there were no significant differences between NOR and HYP at any time. Compared with the pretest, the training increased muscle glycogen content in NOR by ~25% ($P < 0.05$), but this effect was not significantly different from HYP.

## DISCUSSION

It is well established that, within limits of course, training-induced muscle cell adaptation is enhanced as the degree of metabolic stress imposed by the training program is increased. Depending on the specific training goals, metabolic

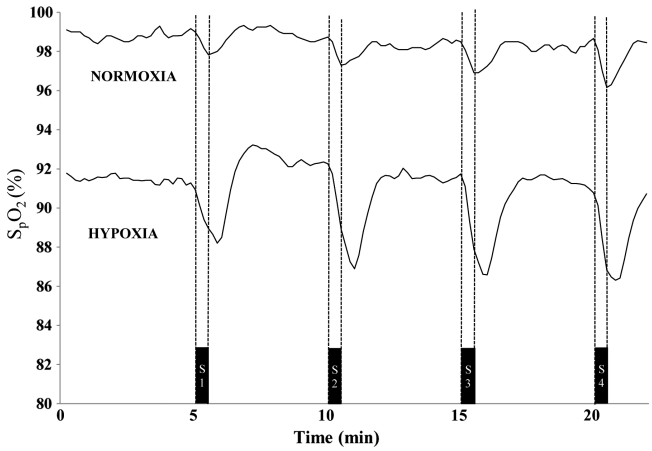


**FIGURE 1—Arterial $O_2$ saturation during SIT in normoxia versus hypoxia. Data represent observations in a preliminary pilot experiment involving an SIT session in young healthy subjects ($n = 10$). Subjects performed four 30-s maximal sprints (S1 to S4) on a cycling ergometer (Avantronic Cyclus 2), interspersed by recovery intervals of 4 min 30 s (cycling at 50 W). During the session, arterial oxygen saturation (%$SpO_2$) was continuously measured at a rate of 60 Hz by pulsoximetry (NellcorN-600-x, Oxismart, Mallinckrodt, St. Louis, MO). The curves represent mean values for an SIT session in normoxia ($F_iO_2$, 20.9%) versus an identical session in hypoxia ($F_iO_2$, 14.5%).**

TABLE 2. Blood lactate and power output during the SIT sessions.

| | | NOR | | HYP | |
|---|---|---|---|---|---|
| | | Week 1 | Week 6 | Week 1 | Week 6 |
| Mean power (W) | | | | | |
| | First sprint | 702 ± 33 | 736 ± 31* | 639 ± 19 | 679 ± 25* |
| | Last sprint | 609 ± 34** | 653 ± 35** | 591 ± 22** | 610 ± 21** |
| Peak power (W) | | | | | |
| | First sprint | 944 ± 52 | 954 ± 35 | 976 ± 45 | 989 ± 28 |
| | Last sprint | 873± 45** | 911 ± 48 | 932 ± 61 | 934 ± 22 |
| Blood lactate ($mmol \cdot L^{-1}$) | | | | | |
| | First sprint | 6.9 ± 0.6 | 7.5 ± 0.5 | 8.1 ± 0.7 | 7.8 ± 0.7 |
| | Last sprint | 13.1 ± 0.7** | 13.7 ± 1.0** | 13.6 ± 0.3** | 14.2 ± 0.4** |
| Peak heart rate ($beats \cdot min^{-1}$) | | | | | |
| | First sprint | 181 ± 4 | 179 ± 3 | 181 ± 3 | 179 ± 4 |
| | Last sprint | 183 ± 3 | 181 ± 3 | 187 ± 4** | 184 ± 3 |

Values are presented as mean ± SEM observations during the first (week 1) and the last week (week 6) of the training period involving 30-s intermittent sprints in either normoxia (NOR, $n = 9$; $F_iO_2$, 20.9%) or hypoxia (HYP, $n = 10$; $F_iO_2$, 14.5%). The number of sprints per session was increased from four in week 1 to nine in week 6. Peak heart rate, mean, and peak power outputs during the first and the last sprints in week 1 and week 6 are given. Blood lactate was measured 3 min after the first and the last sprint.
*$P < 0.05$ versus week 1.
**$P < 0.05$ versus first sprint.

stress in endurance training can be raised either by increasing the duration and/or intensity of the exercise sessions or by doing training sessions in a carbohydrate-depleted or fasted state (34). Another strategy is to exercise in a state of reduced oxygen allowance in either hypobaric (~altitude) or normobaric hypoxia (~simulated altitude) (29). We postulated that low-volume SIT in hypoxia is more potent than SIT in normoxia to enhance endurance exercise performance due to more explicit beneficial physiological adaptations to facilitate aerobic and anaerobic energy turnover in skeletal muscles. In addition, the capacity to perform short high-intensity anaerobic exercise is unaffected by acute exposure to hypoxia, which allows athletes to maintain normal training intensities (38). Therefore, we compared the effects of a 6-wk SIT program involving 30-s sprints interspersed by recovery intervals of 4 min 30 s in normoxia (NOR) versus similar training in hypoxia (NOR; 14.5% $F_iO_2$, corresponding to ~3000 m altitude) on $\dot{V}O_{2max}$ and time trial performance in both normoxia and hypoxia, as well as on some muscular metabolic characteristics. As expected, peak and mean workloads during training were similar between the two training groups (Table 2). Nonetheless, SIT in hypoxia, but not SIT in normoxia, increased muscle PFK activity as well as slightly elevated functional power output at the 4-mmol lactate threshold (LT4). However, in the conditions of the current study, these beneficial physiological adaptations translated into performance enhancement neither in a 10-min simulated time trial nor in an incremental $\dot{V}O_{2max}$ test in either normoxia or in hypoxia.

As indicated earlier, part of the rationale driving the current study is that the capacity to perform short bouts of anaerobic exercise is intact during acute exposure to moderate hypoxia (38). This is explained by the fact that increased fraction of anaerobic ATP production via glycolysis adequately compensates for the hypoxia-induced drop of oxidative energy turnover. Hence, compared with normoxia, stress on glycolytic flux is significantly increased, which conceivably may stimulate the up-regulation of this energy pathway. Indeed, it is well established that consistent high-intensity exercise training involving high fraction of anaerobic ATP production via glycolysis stimulates muscle PFK activity (16,20). Consistent with such assumptions, we found 6 wk of SIT in hypoxia to increase muscle PFK activity approximately threefold more than an equivalent volume of SIT in normoxia (Fig. 2). This finding is in line with earlier reports showing high-intensity endurance training in hypoxia to cause greater up-regulation of muscle PFK mRNA expression than similar training in normoxia (35,40). This effect probably is at least partly due to elevated HIF-1$\alpha$ activity during training in hypoxia. HIF-1$\alpha$ is suggested to stimulate transcription of the PFK enzyme (37). In addition, elevated HIF-1$\alpha$ mRNA abundance induced by hypoxic endurance training also has been found to be associated with higher PFK protein expression (35,40).

The bulk of ATP production during a 30-s all-out sprint is accounted for by muscle phosphocreatine breakdown plus anaerobic glycogen degradation to lactate (31). However, during repeated sprints interspersed by short rest intervals, the reactivation of glycogenolysis via allosteric activation of the phosphorylase enzyme becomes gradually impaired as the number of sprints is increased. This failure to reactivate glycogenolysis is particularly true in type 2 fibers exhibiting at the same time the largest inhibition of $Ca^{2+}$ activation and the most explicit drop of intramyocellular pH due to $H^+$ accumulation (31).

This important role of oxidative metabolism in maintaining power output during SIT definitely partly explains the potential of SIT to stimulate muscular oxidative capacity (3,6–8,13,17). Along this line, it is the prevailing opinion that 5′ adenosine monophosphate-activated protein kinase (AMPK) by SIT is implicated in the up-regulation of mitochondrial adaptation. In this regard, some earlier studies have shown that AMPK activity is acutely increased after an SIT session (9,14,18). However, to the best of our knowledge, the effects of consistent SIT on basal muscle AMPK expression are unknown. We did not evaluate the acute effects of exercise, but analyses on resting muscle biopsies showed that the 6-wk SIT period up-regulated neither muscle AMPK total protein content nor AMPK phosphorylation status (~activity). Studies in our (33,34) and other laboratories (27) have previously

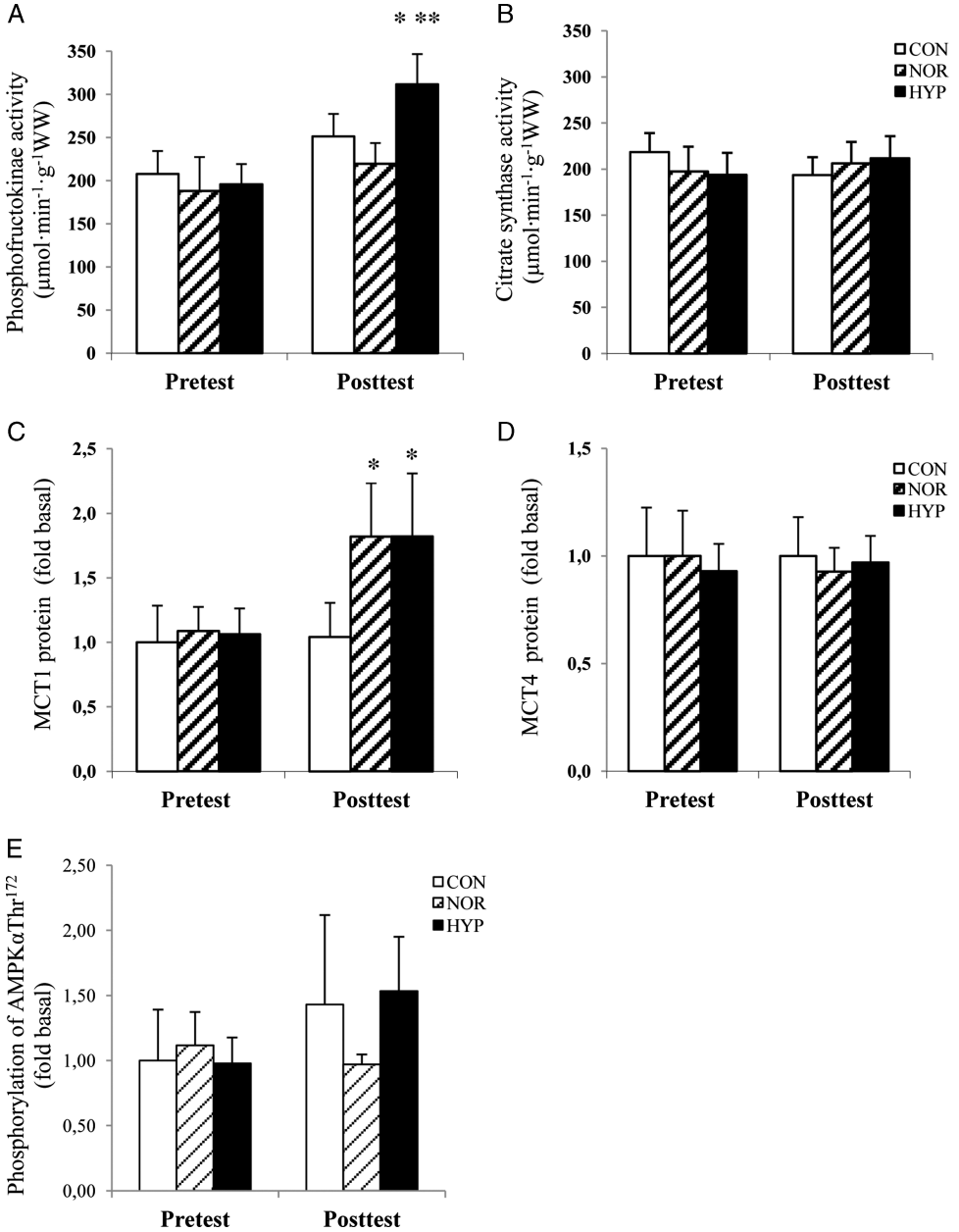


**FIGURE 2—Effect of SIT on muscle PFK (A) and CS activity (B), and on MCT1 (C), MCT4 (D), and AMPK protein content (E). Values are presented as mean ± SEM before (pretest) and after (posttest) the intervention period. Subjects were enrolled in a 6-wk training period involving 30-s intermittent sprints in either normoxia ($n$ = 9; $F_iO_2$, 20.9%) or hypoxia ($n$ = 10; $F_iO_2$, 14.5%). The control group ($n$ = 10) did not train. In both the pretest and the posttest, a needle biopsy was taken from resting musculus vastus lateralis. For the AMPK data (E), a smaller number of observations was included (CON: $n$ = 7; NOR: $n$ = 6; HYP: $n$ = 7). WW, wet weight. See Methods section for more details. *$P$ < 0.05 versus pretest, *$P$ < 0.05 versus NOR posttest.**

shown high-volume endurance training readily to increase AMPK abundance in muscle. Conversely, studies that looked at the effects of low-volume high-intensity endurance training have yielded equivocal results, with two studies showing unchanged AMPK activity posttraining (10,39) versus one study reporting slightly increased AMPK activity posttraining (1). In this study, we added the observation that even lower volumes of SIT failed to affect basal muscle AMPK. Thus, literature data taken together with the current findings clearly indicate that high-volume endurance training is probably more effective than low-volume high-intensity training to stimulate AMPK abundance in muscle. Consistent with unchanged AMPK, SIT even in normoxia did not elevate CS activity. This is in conflict with earlier reports showing substantial increase in markers of oxidative capacity in muscles post-SIT, including increase of CS activity (3,6–8,13,17), 3-hydroxyacyl CoA dehydrogenase (7,17), pyruvate dehydrogenase (7), cytochrome *c* oxidase (13), and succinate dehydrogenase (28). The reason for this discrepant finding is unclear but may be due to the late timing of the muscle

TABLE 3. Effects of SIT during the incremental exercise tests in normoxia and hypoxia.

| | CON | | NOR | | HYP | |
|---|---|---|---|---|---|---|
| | Pretest | Posttest | Pretest | Posttest | Pretest | Posttest |
| LT4 (W) | | | | | | |
| $MAX_{nor}$ | 228 ± 13 | 225 ± 12 | 219 ± 16 | 220 ± 13 | 206 ± 9 | 221 ± 5*,** |
| $MAX_{hyp}$ | 193 ± 7 | 190 ± 10 | 173 ± 11 | 180 ± 12 | 173 ± 8 | 189 ± 6*,** |
| Time to exhaustion (min) | | | | | | |
| $MAX_{nor}$ | 25.4 ± 1.4 | 25.0 ± 1.4 | 24.4 ± 1.2 | 25.1 ± 0.9** | 24.0 ± 0.8 | 25.2 ± 0.6*,** |
| $MAX_{hyp}$ | 21.6 ± 1.1 | 21.9 ± 1.4 | 20.2 ± 0.7 | 21.4 ± 0.7* | 20.1 ± 0.5 | 21.7 ± 0.6*,** |
| Peak $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$) | | | | | | |
| $MAX_{nor}$ | 54.8 ± 2.3 | 54.1 ± 1.7 | 56.8 ± 3 | 60.1 ± 2.6* | 55.1 ± 1.7 | 59.2 ± 2.2*,** |
| $MAX_{hyp}$ | 48.7 ± 1.9 | 45.5 ± 1.9* | 50.9 ± 2.8 | 49.8 ± 2.8** | 48.5 ± 1.6 | 48.3 ± 1.7** |
| Peak heart rate (beats $min^{-1}$) | | | | | | |
| $MAX_{nor}$ | 186 ± 2.5 | 186 ± 2.1 | 190 ± 3.5 | 192 ± 3.07 | 192 ± 3.3 | 192 ± 3.1 |
| $MAX_{hyp}$ | 181 ± 2.3 | 180 ± 2.2 | 186 ± 2.9 | 187 ± 3.0 | 186 ±3.8 | 187 ± 2.9 |
| Peak blood lactate ($mmol \cdot L^{-1}$) | | | | | | |
| $MAX_{nor}$ | 12.6 ± 0.7 | 12.7 ± 0.8 | 12.6 ± 0.7 | 13.6 ± 0.9 | 13.3 ± 0.4 | 14.6 ± 0.8 |
| $MAX_{hyp}$ | 12.5 ± 0.8 | 13.5 ± 0.9 | 12.9 ± 0.8 | 14.3 ± 0.9 | 13.2 ± 0.5 | 14.4 ± 0.5 |
| Peak RER | | | | | | |
| $MAX_{nor}$ | 1.01 ± 0.02 | 1.01 ± 0.03 | 1.07 ± 0.03 | 1.03 ± 0.02 | 1.09 ± 0.03 | 1.06 ± 0.03 |
| $MAX_{hyp}$ | 1.02 ± 0.04 | 1.01 ± 0.01 | 1.01 ± 0.02 | 1.02 ± 0.02 | 1.02 ± 0.02 | 1.01 ± 0.01 |

Values are presented as mean ± SEM before (pretest) and after (posttest) the intervention period. Subjects were enrolled in a 6-wk training period involving 30-s intermittent sprints in either normoxia (NOR, $n = 9$; $F_iO_2$, 20.9%) or hypoxia (HYP, $n = 10$; $F_iO_2$, 14.5%). The control group (CON, $n = 10$) did not train. In both the pretest and the posttest, the subjects performed a maximal incremental exercise test in normoxia ($MAX_{nor}$) and in hypoxia ($MAX_{hyp}$) with an interval of 2 d.

*$P < 0.05$ versus pretest.

**$P < 0.05$ versus CON.

LT4, 4-mmol lactate threshold.

biopsying in the posttest, which may have masked a transient increase in either AMPK and/or CS activity after the last training session. Nonetheless, power output at the 4-mmol lactate threshold in both normoxia and hypoxia was slightly increased after SIT in hypoxia but not after SIT in normoxia (Table 3). The up-regulation of muscular oxidative capacity resulting in less lactate production for a given submaximal power output (W) is the obvious mechanism underlying this right shift of the lactate curve in the conditions of the current study. In this regard, it is also interesting to note that mean power outputs during the SIT sessions were similar between normoxia and hypoxia from the start to the end of the training program. This indicates that even during SIT in hypoxia, within training sessions, oxidative energy pathways were able to adequately compensate for the drop of glycogenolytic activity from the first to the last sprint (Table 2).

During high-intensity "anaerobic" exercise, muscle lactate production is markedly enhanced, most prominently in the active type II motor units. Part of the lactate so formed is exported from muscle cells via the MCT1 and the MCT4 (15). We expected that SIT in hypoxia, due to greater fraction of energy production during the sprints coming from glycogen degradation to lactate, would be more effective to increase muscle MCT content than SIT in normoxia. However, contrary to our expectations, blood lactate levels during SIT were similar between normoxia and hypoxia (Table 2), which probably eliminated the potential stimulus for differential training adaptation between groups. Accordingly, SIT increased muscle MCT1 protein content in either group by ~70%, whereas MCT4 content was constant between the pretest and the posttest. Our current findings are consistent with earlier studies showing training readily to increase muscle MCT1 content. However, equivocal findings have been reported with regard to the effects of training on MCT4 with some studies showing no change (4,24) versus others reporting a slight increase (5,25) after a short period of high-intensity training. However, the role of MCT1 in muscle lactate metabolism is probably more important than the role of MCT4 because MCT1 protein abundance, but not MCT4 expression, is inversely related to rate of fatigue development in short maximal exercise (32). Furthermore, MCT1 also plays an important role in lactate import and oxidation in mitochondria (12). However, in the current study, we did not separate the mitochondrial from the sarcolemmal MCT1 fraction.

An important aim of the present study was to evaluate whether SIT in hypoxia would be more successful than SIT in normoxia to boost endurance exercise performance. We evaluated endurance performance using a 10-min simulated cycling time trial. However, irrespective of whether SIT sessions were performed in normoxia or hypoxia, the training intervention improved performance by approximately 6%–8%, both in the time trial in normoxia and in hypoxia. Exercise intensity in a time trial as short as 10 min is very close to $\dot{V}O_2$ max and involves a substantial energy contribution from glycolysis as evidenced by blood lactate levels higher than 10 $mmol \cdot L^{-1}$ postexercise. The similar performance improvement in HYP and NOR is compatible with similar elevation of $\dot{V}O_{2max}$ posttraining, even against the face of higher LT4 in the former. In fact, the minor increment of LT4 in HYP conceivably was too small to translate into improved performance in a 10-min time trial. Furthermore, it is also important to point out that time trials were performed immediately after the incremental $\dot{V}O_{2max}$ tests, which conceivably may have impacted performance and variation between subjects due to combination of physiological and mental fatigue. Still, it is reasonable to postulate that the elevated glycolytic capacity (~PFK activity) found in muscles in HYP should have resulted in better time trial performance due to higher anaerobic capacity. Contrary to

TABLE 4. Effects of SIT on performance during the 10-min time trial in normoxia and hypoxia.

| | CON | | NOR | | HYP | |
|---|---|---|---|---|---|---|
| | Pretest | Posttest | Pretest | Posttest | Pretest | Posttest |
| Mean power (W) | | | | | | |
| $TT_{nor}$ | 278 ± 20 | 277 ± 18 | 258 ± 16 | 275 ± 13** | 254 ± 6 | 268 ± 6** |
| $TT_{hyp}$ | 233 ± 13 | 236 ± 15 | 219 ± 11 | 237 ± 9* | 215 ± 6 | 230 ± 8* |
| Peak heart rate (beats·min$^{-1}$) | | | | | | |
| $TT_{nor}$ | 183 ± 3 | 182 ± 3 | 188 ± 4 | 189 ± 2 | 189 ± 3 | 188 ± 4 |
| $TT_{hyp}$ | 181 ± 3 | 178 ± 3 | 183 ± 3 | 183 ± 2 | 183 ± 3 | 182 ± 3 |
| Peak lactate (mmol·L$^{-1}$) | | | | | | |
| $TT_{nor}$ | 10.4 ± 0.7 | 9.9 ± 0.5 | 9.7 ± 1.0 | 11.6 ± 0.8** | 10.2 ± 0.4 | 10.2 ± 0.9 |
| $TT_{hyp}$ | 11.5 ± 0.5 | 11.7 ± 0.6 | 10.4 ± 1.0 | 13.1 ± 0.7* | 10.0 ± 0.9 | 11.7 ± 1.0* |

Values are presented as mean ± SEM before (pretest) and after (posttest) the intervention period. Subjects were enrolled in a 6-wk training period involving 30-s intermittent sprints in either normoxia (NOR, $n = 9$; $F_iO_2$, 20.9%) or hypoxia (HYP, $n = 10$; $F_iO_2$, 14.5%). The control group (CON, $n = 10$) did not train. In both the pretest and the posttest, the subjects performed a 10-min simulated time trial in normoxia ($TT_{nor}$) and in hypoxia ($TT_{hyp,}$) with an interval of 2 d.
*$P < 0.05$ versus pretest.
**$P < 0.05$ versus CON.

this assumption, maximal lactate concentrations achieved during the training sessions (Table 2), the incremental exercise tests (Table 3), as well as the time trials (Table 4) were identical between the groups, indicating similar anaerobic capacity. PFK activity is inhibited by increase in [$H^+$]. Thus, for higher glycolytic potential to result in elevated anaerobic capacity, increased buffer capacity is required not only to prevent premature inhibition of PFK but also to postpone the role of $H^+$ in other intracellular mechanism implicated in the development of muscle fatigue (2).

Initial muscle glycogen content is an important determinant of performance in endurance events lasting more than 60 min (26). Previous studies have demonstrated that low-volume SIT is evenly effective than much higher volumes of steady-state endurance training to elevate muscle glycogen content (7,13,17). Accordingly, 6 wk of SIT raised muscle glycogen content by ~15%–25% independent of whether the training was performed in normoxia or hypoxia.

In conclusion, an increasing number of athletes are performing endurance training sessions in hypoxia with the specific purpose to enhance performance in endurance competitions either at sea level or at altitude. The present study clearly demonstrates that high-intensity SIT in hypoxia is more potent than identical workloads in normoxia to enhance power output at LT4. Furthermore glycolytic enzyme activity was up-regulated after training in hypoxia but not in normoxia. However, in the conditions of the present study, these beneficial physiological adaptations improved endurance exercise performance neither in a normoxic nor in a hypoxic environment.

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## REFERENCES

1. Abbiss CR, Karagounis LG, Laursen PB, et al. Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. *J Appl Physiol*. 2011;110(5):1248–55.
2. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*. 2008;88(1):287–332.
3. Barnett C, Carey M, Proietto J, Cerin E, Febbraio MA, Jenkins D. Muscle metabolism during sprint exercise in man: influence of sprint training. *J Sci Med Sport*. 2004;7(3):314–22.
4. Bickham DC, Bentley DJ, Le Rossignol PF, Cameron-Smith D. The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. *Eur J Appl Physiol*. 2006; 96(6):636–43.
5. Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, Gibala MJ. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(5):R1970–6.
6. Burgomaster KA, Heigenhauser GJ, Gibala MJ. Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. *J Appl Physiol*. 2006;100(6):2041–7.
7. Burgomaster KA, Howarth KR, Phillips SM, et al. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol*. 2008; 586(1):151–60.
8. Burgomaster KA, Hughes SC, Heigenhauser GJ, Bradwell SN, Gibala MJ. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol*. 2005;98(6):1985–90.
9. Chen ZP, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE. AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. *Am J Physiol Endocrinol Metab*. 2000;279(5):E1202–6.
10. Clark SA, Chen ZP, Murphy KT, et al. Intensified exercise training does not alter AMPK signaling in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2004;286(5):E737–43.
11. den Hoed M, Hesselink MKC, van Kranenburg GPJ, Westerterp KR. Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity. *J Appl Physiol*. 2008; 105(2):561–8.
12. Dubouchaud H, Butterfield GE, Wolfel EE, Bergman BC, Brooks GA. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;278(4):E571–9.
13. Gibala MJ, Little JP, van EM, et al. Short-term sprint interval versus traditional endurance training: similar initial adaptations in

human skeletal muscle and exercise performance. *J Physiol*. 2006; 575(Pt 3):901–11.
14. Gibala MJ, McGee SL, Garnham AP, Howlett KF, Snow RJ, Hargreaves M. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol*. 2009;106(3):929–34.
15. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol*. 2004;558(Pt 1):5–30.
16. Jacobs I, Esbjornsson M, Sylven C, Holm I, Jansson E. Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate. *Med Sci Sports Exerc*. 1987;19(4):368–74.
17. Laursen PB, Jenkins DG. The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. *Sports Med*. 2002;32(1):53–73.
18. Little JP, Safdar A, Bishop D, Tarnopolsky MA, Gibala MJ. An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1alpha and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(6):R1303–10.
19. Lowry OH, Passoneau JV. *A Flexible System of Enzymatic Analysis*. New York: Academic Press, 1972.
20. MacDougall JD, Hicks AL, MacDonald JR, McKelvie RS, Green HJ, Smith KM. Muscle performance and enzymatic adaptations to sprint interval training. *J Appl Physiol*. 1998;84(6):2138–42.
21. Macpherson RE, Hazell TJ, Olver TD, Paterson DH, Lemon PW. Run sprint interval training improves aerobic performance but not maximal cardiac output. *Med Sci Sports Exerc*. 2011;43(1):115–22.
22. Melissa L, MacDougall JD, Tarnopolsky MA, Cipriano N, Green HJ. Skeletal muscle adaptations to training under normobaric hypoxic versus normoxic conditions. *Med Sci Sports Exerc*. 1997; 29(2):238–43.
23. Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak performance. *Sports Med*. 2010; 40(1):1–25.
24. Mohr M, Krustrup P, Nielsen JJ, et al. Effect of two different intense training regimens on skeletal muscle ion transport proteins and fatigue development. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292(4):R1594–602.
25. Perry CG, Heigenhauser GJ, Bonen A, Spriet LL. High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Appl Physiol Nutr Metab*. 2008;33(6):1112–23.
26. Philp A, Hargreaves M, Baar K. More than a store: regulatory roles for glycogen in skeletal muscle adaptation to exercise. *Am J Physiol Endocrinol Metab*. 2012;302(11):E1343–51.
27. Richter EA, Ruderman NB. AMPK and the biochemistry of exercise: implications for human health and disease. *Biochem J*. 2009;418(2):261–75.
28. Saltin B, Nazar K, Costill DL, et al. The nature of the training response; peripheral and central adaptations of one-legged exercise. *Acta Physiol Scand*. 1976;96(3):289–305.
29. Schmutz S, Dapp C, Wittwer M, et al. A hypoxia complement differentiates the muscle response to endurance exercise. *Exp Physiol*. 2010;95(6):723–35.
30. Smith KJ, Billaut F. Influence of cerebral and muscle oxygenation on repeated-sprint ability. *Eur J Appl Physiol*. 2010;109(5): 989–99.
31. Spriet LL, Lindinger MI, McKelvie RS, Heigenhauser GJ, Jones NL. Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *J Appl Physiol*. 1989;66(1):8–13.
32. Thomas C, Perrey S, Lambert K, Hugon G, Mornet D, Mercier J. Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans. *J Appl Physiol*. 2005;98(3):804–9.
33. Van Proeyen K, De Bock K, Hespel P. Training in the fasted state facilitates re-activation of eEF2 activity during recovery from endurance exercise. *Eur J Appl Physiol*. 2011;111(7):1297–305.
34. Van Proeyen K, Szlufcik K, Nielens H, Ramaekers M, Hespel P. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *J Appl Physiol*. 2011;110(1):236–45.
35. Vogt M, Puntschart A, Geiser J, Zuleger C, Billeter R, Hoppeler H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J Appl Physiol*. 2001;91(1): 173–82.
36. Wehrlin JP, Hallen J. Linear decrease in $\dot{V}O_{2max}$ and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol*. 2006;96(4):404–12.
37. Wenger RH. Cellular adaptation to hypoxia: $O_2$-sensing protein hydroxylases, hypoxia-inducible transcription factors, and $O_2$-regulated gene expression. *FASEB J*. 2002;16(10):1151–62.
38. Weyand PG, Lee CS, Martinez-Ruiz R, Bundle MW, Bellizzi MJ, Wright S. High-speed running performance is largely unaffected by hypoxic reductions in aerobic power. *J Appl Physiol*. 1999; 86(6):2059–64.
39. Yeo WK, Paton CD, Garnham AP, Burke LM, Carey AL, Hawley JA. Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *J Appl Physiol*. 2008;105(5):1462–70.
40. Zoll J, Ponsot E, Dufour S, et al. Exercise training in normobaric hypoxia in endurance runners. III. Muscular adjustments of selected gene transcripts. *J Appl Physiol*. 2006;100(4):1258–66.