

## Experimental Physiology – Research Paper

# Training in hypoxia fails to further enhance endurance performance and lactate clearance in well-trained men and impairs glucose metabolism during prolonged exercise

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The aim of this study was to investigate the synergistic effects of endurance training and hypoxia on endurance performance in normoxic and hypoxic conditions (~3000 m above sea level) as well as on lactate and glucose metabolism during prolonged exercise. For this purpose, 14 well-trained cyclists performed 12 training sessions in conditions of normobaric hypoxia (HYP group,  $n = 7$ ) or normoxia (NOR group,  $n = 7$ ) over 4 weeks. Before and after training, lactate and glucose turnover rates were measured by infusion of exogenous lactate and stable isotope tracers. Endurance performance was assessed during incremental tests performed in normoxia and hypoxia and a 40 km time trial performed in normoxia. After training, performance was similarly and significantly improved in the NOR and HYP groups (training,  $P < 0.001$ ) in normoxic conditions. No further effect of hypoxic training was found on markers of endurance performance in hypoxia (training  $\times$  hypoxia interaction, n.s.). In addition, training and hypoxia had no significant effect on lactate turnover rate. In contrast, there was a significant interaction of training and hypoxia ( $P < 0.05$ ) on glucose metabolism, as follows: plasma insulin and glucose concentrations were significantly increased; glucose metabolic clearance rate was decreased; and the insulin to glucagon ratio was increased after training in the HYP group. In conclusion, our results show that, compared with training in normoxia, training in hypoxia has no further effect on endurance performance in both normoxic and hypoxic conditions or on lactate metabolic clearance rate. Additionally, these findings suggest that training in hypoxia impairs blood glucose regulation in endurance-trained subjects during exercise.

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Living and training at sea level with the addition of key training sessions in hypoxia [the so-called live low–train high (LLTH) method] is used by athletes to increase endurance performance abilities at sea level and at altitude (Wilber, 2007). However, the efficiency of LLTH is debated; some studies have reported enhanced sea-level endurance performance (Hoppeler *et al.* 2003; Dufour *et al.* 2006) and improved cardiorespiratory (Terrados *et al.* 1988; Hendriksen & Meeuwse, 2003) or muscle performance (Hoppeler & Vogt, 2001; Vogt *et al.* 2001), whereas other studies showed no benefits when compared with training in normoxia (Truijens *et al.* 2003; Ventura *et al.* 2003; Roels *et al.* 2005, 2007a; Bonetti & Hopkins, 2009). Furthermore,

the effect of LLTH on endurance performance at altitude is also unclear (Hoppeler *et al.* 2008).

Nevertheless, LLTH appears to effectively produce multiple metabolic adaptations in skeletal muscle (Hoppeler & Vogt, 2001; Vogt *et al.* 2001; Zoll *et al.* 2006). Specifically, 3–6 weeks of LLTH training has been shown to induce changes related to mitochondrial function (Ponsot *et al.* 2006; Roels *et al.* 2007b) and biogenesis (Zoll *et al.* 2006) as well as modifications of glycolytic enzyme gene expression (Zoll *et al.* 2006) and activity (Terrados *et al.* 1988). In addition, a recent study on previously untrained individuals reported that LLTH resulted in greater improvement of glucose homeostasis and blood

lipid profile than training in normoxia, indicating a potent effect of hypoxic training on metabolic risk factors (Haufe *et al.* 2008). Therefore, hypoxia may potentiate the effect of exercise training on glucose homeostasis independently of training status. To our knowledge, however, no study has reported the effect of LLTH on glucose kinetics in trained individuals, who should have a high insulin sensitivity.

In addition to glucose metabolism variables, the gene expression of muscle lactate transport protein monocarboxylate 1 (MCT1) has also been reported to be increased after LLTH training (Zoll *et al.* 2006). At the muscle level, lactate transport across the sarcolemma appears to be related to the abundance of MCT1 and monocarboxylate 4 (MCT4; Dubouchaud *et al.* 2000; Thomas *et al.* 2005; Bonen, 2006), and exercise training is known to increase MCT1 and MCT4 muscle protein content (Dubouchaud *et al.* 2000; Thomas *et al.* 2005; Burgomaster *et al.* 2007). Both proteins are known to allow cell-to-cell and intracellular lactate shuttles, hence, lactate oxidation (Dubouchaud *et al.* 2000; Hashimoto & Brooks, 2008). Endurance training alone has been shown to increase lactate clearance and oxidation (Donovan & Brooks, 1983; Stanley *et al.* 1985; MacRae *et al.* 1995; Phillips *et al.* 1995; Bergman *et al.* 1999), while endogenous lactate production may remain unchanged (Donovan & Brooks, 1983) or decrease (Bergman *et al.* 1999). While the relationship between muscle lactate transport and exercise performance is unclear (Bonen, 2006; Bentley *et al.* 2009), endurance performance has been reported to be related to whole-body blood lactate kinetics (Messonnier *et al.* 1997, 2001, 2002; Hawley, 2002; Thomas *et al.* 2004). Thus, lactate kinetics may be a good marker for the effects of endurance training.

Therefore, we hypothesized that hypoxia has synergistic effects with endurance training. If this hypothesis is true, then training in hypoxia would result in greater improvements of endurance performance, both at sea level and at altitude, and a greater increase of whole-body lactate turnover rate.

Here, we tested the additive effects of high-intensity training and hypoxia on endurance performance in normoxic (~550 m above sea level) and hypoxic conditions (~3000 m). Lactate turnover rate and glucose kinetics were investigated during prolonged exercise using exogenous lactate and infusion of stable isotope tracers.

## Methods

### Participants

Fourteen male well-trained competitive cyclists and triathletes (height,  $177 \pm 2$  cm; body mass,  $70.4 \pm 1.3$  kg; body fat,  $12 \pm 1\%$ ; and age,  $29 \pm 1$  years) volunteered to participate in this study. Inclusion criteria were the following: aged between 18 and 40 years; extensive

endurance training for more than 3 years; and maximal oxygen uptake ( $\dot{V}_{O_{2max}} > 60$  ml min<sup>-1</sup> kg<sup>-1</sup>). In addition, volunteers were training for at least 6 h per week and be in a baseline endurance training cycle at the start of the experiment. All subjects were fully informed of the risks associated with participation in the study protocol and gave written informed consent. The study conformed to the recommendations of the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Biology and Medicine of the University of Lausanne, Switzerland. Subjects were asked to avoid any exposure to an altitude of more than 2000 m during the study protocol.

### Experimental design

Volunteers were randomly assigned to one of two treatment groups: a control group ( $n = 7$ ) training in normoxia (NOR); and a group ( $n = 7$ ) training in normobaric hypoxia (HYP). The experimental protocol consisted of four pretraining baseline performance and metabolic tests (PRE), a 5 week supervised training period, and four post-training performance and metabolic tests (POST). In total, each subject made 20 visits to the laboratory over 8–9 weeks.

### Training programme

The training period consisted of 4 weeks of supervised training at the laboratory and 1 week of reduced training volume and intensity as an active recovery week. During the 4 weeks of training, three sessions per week were performed at the laboratory under supervision. In addition, subjects were asked to perform their usual base/endurance training at home and to have at least 1 day of complete rest each week. Additionally, they were asked to avoid any high-intensity interval training, with the exception of the high-intensity training performed at the laboratory. Participants were provided with heart-rate monitors (Polar RS400, Polar Electro Oy, Kempele, Finland) and were asked to record all training sessions performed outside the laboratory. Training volumes and intensities were then checked by downloading training data from the heart-rate monitors.

**Supervised training.** Two high-intensity interval training (HIT) sessions and one basic endurance session were planned each week. All training sessions were performed on the individual road bikes belonging to the volunteers. Exercise intensity was monitored continuously and displayed by means of power measurement devices (Powertap SL, Saris Cycling Group, Madison, WI, USA). The first HIT session consisted of short (30 s to 2 min) cycling intervals performed at subjects' normoxic or

**Table 1. Summary of the training programme performed at the laboratory under supervision**

Week	Session 1: HIT	Target intensity		
		NOR: MAP HYP: Hypoxic MAP	NOR: $P_{VT2}$ HYP: Hypoxic $P_{VT2}$	NOR: $<P_{VT1}$ HYP: $<$ Hypoxic $P_{VT1}$
			Session 2: HIT	Session 3: Cont
1	8 × 2 min at 100% MAP/66 min		5 × 6 min at VT2/72 min	100 min at 80–90% VT1
2	6 × 2 min at 100% MAP, 6 × 1 min at 110% MAP/69 min		3 × 10 min at VT2/80 min	100 min at 80–90% VT1
3	6 × 2 min at 100% MAP, 8 × 1 min at 110% MAP/81 min		4 × 10 min at VT2/85 min	100 min at 80–90% VT1
4	6 × 2 min at 100% MAP, 6 × 1 min at 110% MAP, 8 × 30 s at 120% MAP/83 min		4 × 12 min at VT2/93 min	100 min at 80–90% VT1

Values are expressed as time (min) spent at high intensity over the duration (min) of the session. Abbreviations: HIT, high-intensity interval training session; NOR, group training in normoxia; HYP, group training in hypoxia; Cont, continuous basic endurance training; MAP, maximal aerobic power; VT1 and VT2, ventilatory thresholds 1 and 2, respectively; and  $P_{VT1}$  and  $P_{VT2}$ , power output associated with the first and the second ventilatory threshold, respectively.

hypoxic maximal aerobic power (MAP). The second HIT session consisted of longer intervals, lasting 6–12 min, performed at each subject's respective second ventilatory threshold (VT2), as determined during the incremental tests (see the 'Exercise performance tests' section). Before and after each HIT session, 15 min of light- to moderate-intensity cycling exercise was performed to warm-up and cool-down. The amount of time spent at high intensity (i.e. at or above VT2 and MAP) was increased each week (Table 1). After 2 weeks, power output at VT2 was increased by 10%. The third session (continuous cycling) remained identical throughout the protocol. This session consisted of continuous cycling at an intensity corresponding to 80–100% of the power output associated with the first ventilatory threshold (VT1) preceded by 5 min of warm-up. During these three sessions, the NOR group breathed room air, while the HYP group breathed a hypoxic gas mixture simulating an altitude of ~3000 m. The simulated altitude of 3000 m was chosen because it is the median altitude used in the above-mentioned studies reporting the effect of a LLTH training protocol. Over the 4 weeks of training, 17.2 h of supervised training were performed in the laboratory.

Room temperature was maintained at 20–22°C, and volunteers were cooled by a fan placed in front of them. To ensure proper hydration and sufficient carbohydrate intake during exercise, subjects were provided with an electrolyte-carbohydrate sports drink (Isotonic, Sponser, Wollerau, Switzerland) *ad libitum*. At the end of each HIT session, subjects were encouraged to ingest a carbohydrate-protein drink and/or a carbohydrate-rich bar (Sponser, Wollerau, Switzerland) to optimize muscle glycogen recovery.

Data recorded by the power output measurement devices were stored on a computer for further quantification of the training load (see 'Calculations').

**Active recovery week.** The fifth week of the training period was low volume, with low- to moderate-intensity training. Volunteers were advised to train for 6 h during

this week, mainly below the heart rate associated with VT1. A short HIT session was performed on the Wednesday of this week.

**Exercise performance tests:  $\dot{V}_{O_{2max}}$  in normoxia and hypoxia, and time trial (TT).** During the first test session, body composition,  $\dot{V}_{O_{2max}}$  and MAP were assessed. A preliminary time-trial performance test was then performed.

Upon arrival at the laboratory, height and body mass of the subjects were measured, and their body fat mass was estimated using the four-skinfold thickness measurement method.

Thereafter,  $\dot{V}_{O_{2max}}$  and MAP were measured during an incremental workload test performed on an electronically braked cycle ergometer (Ergoline 400, GE Medical Systems, USA). During this test, oxygen uptake ( $\dot{V}_{O_2}$ ) and carbon dioxide production ( $\dot{V}_{CO_2}$ ) were measured breath by breath by an automated indirect calorimeter (Vmax Spectra, SensorMedics, Yorba Linda, CA, USA), which was calibrated immediately before each test with gases of known concentrations and a 3 l syringe. Heart rate was continuously recorded and averaged every 5 s by a commercially available heart rate monitor (Polar S810, Polar Electro Oy). Heart rate and ventilatory data were synchronized offline. After 3 min of rest, subjects began pedalling at an initial power output of 60 W for 5 min. Thereafter, workload increased by 35 W every 3 min until exhaustion. The  $\dot{V}_{O_{2max}}$  was considered as the highest 30 s average of  $\dot{V}_{O_2}$ . During the entire test, oxyhaemoglobin (HbO<sub>2</sub>) saturation was monitored by fingertip pulse oxymetry (Omeda, Datex, GE Healthcare). Thirty seconds before the end of each step, ratings of perceived exertion (RPE), according to the Borg 6–20 scale, and HbO<sub>2</sub> saturation were recorded. The MAP was defined as the power output of the last completed step plus the fraction of the non-competed step multiplied by 35 W if the time spent in the latter was greater than 30 s. From the MAP value, the work to be performed during the TT ( $W_{TT}$ ) was then calculated, as previously described (Jeukendrup *et al.*

1996), according to the following formula:

$$W_{\text{TT}} = 0.75 \times 3600 \times \text{MAP} \quad (1)$$

Minute ventilation ( $\dot{V}_E$ ),  $\dot{V}_{\text{O}_2}$ ,  $\dot{V}_{\text{CO}_2}$  and heart rate were averaged every 15 s and plotted against time to determine the power output and heart rate at the first (VT1) and second ventilatory threshold (VT2), which were subsequently used for establishment of each subject's training plan (Table 1). The VT1 and VT2 were determined, according to standard criteria (Faria *et al.* 2005), by two independent investigators.

After an active recovery, volunteers were familiarized with the 40 km TT (see the 'Time trial' section for further details).

Two to three days after the first test, volunteers underwent an incremental test to exhaustion in normobaric hypoxic conditions to determine  $\dot{V}_{\text{O}_2\text{max}}$  at a simulated altitude corresponding to  $\sim 3000$  m. The normobaric hypoxic conditions were obtained by dilution of ambient air with nitrogen (Altitrainer 200, SMTec, Nyon, Switzerland). The Altitrainer<sup>®</sup> and the indirect calorimeter were connected by a two-way Y-shaped non-rebreathing valve with a low dead volume (Hans Rudolph, Shawnee, KS, USA).

A similar protocol to that used for determination of  $\dot{V}_{\text{O}_2\text{max}}$  and MAP in normoxic conditions was performed in conditions to simulate an altitude of  $\sim 3000$  m. During this trial, fractional inspired  $\text{O}_2$  was maintained at 14.5%. Submaximal and maximal indicators of performance at altitude were determined according to the same criteria as those described for the  $\dot{V}_{\text{O}_2\text{max}}$  and MAP test in normoxic conditions.

**Time trial.** Two to three days after the previous visit, the subjects reported at 07.30 h to the laboratory following overnight fasting. During the 48 h prior to the test, subjects were asked to avoid caffeine, alcohol and any strenuous exercise. During that period, they were advised to consume a high-carbohydrate diet and to record their dietary intakes.

Upon arrival, the volunteers emptied their bladder and were weighed. Afterwards, they lay on a bed, and a catheter was inserted into an antecubital vein for blood sampling. The catheter was kept patent by infusion of saline during the entire test. An initial blood sample was drawn for measurement of insulin, cortisol, free fatty acids (FFA), lactate, glucose and glucagon concentrations. Baseline haematological variables were also assessed at this time. Thereafter, volunteers sat on their own road bike equipped with a power output measurement hub (Powertap SL, Saris Cycling Group) and placed on a stationary magnetic resistance (Satori, Tacx, Wassenaar, The Netherlands). A 20 min warm-up began at a self-selected intensity. After 10 min of warm-up, the Powertap<sup>®</sup> offset was zeroed

according to the manufacturer's instructions. This device has previously been shown to be accurate and reliable for performance measurements (Gardner *et al.* 2004). During the TT, power output and heart rate were recorded at 1 s intervals by a handlebar computer connected to the power output measurement hub (Cervo, Saris Cycling Group). At the end of the warm-up, a blood sample was drawn, and the TT was started. According to eqn (1), the distance was expressed as mechanical work (Jeukendrup *et al.* 1996) that was to be cycled as fast as possible. To allow volunteers to fine-tune their pace during the trial, they were continuously informed by the handlebar computer of the mechanical work already performed and the amount of mechanical work corresponding to 25, 50, 75, 90, 95 and 100% of total work. Room temperature was kept at 20–22°C, and volunteers were neither disturbed nor encouraged by the investigators. Water was available *ad libitum*, and subjects were cooled by a fan placed in front of them. Finally, subjects were instructed to stop pedalling upon completion of the task for approximately 5–10 s (for precise determination of the end of the TT on the stored data) before beginning a 10 min active recovery period. Blood samples were taken, and elapsed time was measured when subjects reached 25, 50, 75 and 100% of the  $W_{\text{TT}}$ .

### Metabolic test

Two to three days after the previous exercise test, subjects reported at 07.30 h to the laboratory after fasting overnight for determination of lactate and glucose turnover rates during prolonged exercise. Dietary recommendations were identical to those described for the time trial. In addition, subjects were asked to avoid foods naturally enriched in  $^{13}\text{C}$  during the 48 h preceding the test. The procedures, derived from previous studies performed at rest (Chiolero *et al.* 2000; Revelly *et al.* 2005), are summarized in Fig. 1.

Upon arrival, volunteers emptied their bladder and were then weighed. Thereafter, they lay on a bed, and a catheter was inserted into an antecubital vein of the left arm for blood sampling. An initial blood sample was drawn for baseline determination of plasma insulin, glucagon, cortisol, glucose and lactate concentrations. Expired air samples were then collected in quadruplicate (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA) for the assessment of baseline expired air  $^{13}\text{CO}_2$  abundance. Thereafter, a second catheter was inserted into the antecubital vein of the right arm for stable isotope tracer infusion.

Ten minutes before the start of exercise, a priming ( $11 \mu\text{mol kg}^{-1}$ ) constant infusion of  $0.11 \mu\text{mol kg}^{-1} \text{min}^{-1}$  6,6- $^2\text{H}_2$ -glucose began, and a 10 min baseline measurement of  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  at rest was performed. Subjects then mounted the cycle ergometer,

the 6,6-<sup>2</sup>H<sub>2</sub>-glucose infusion rate was increased to 0.44 μmol kg<sup>-1</sup> min<sup>-1</sup>, and subjects started pedalling at an intensity of 35% of MAP. After 15 min, exercise intensity was increased to 50% of MAP. Exercise intensity then remained constant until 180 min. After 40 min of exercise, a second blood sample was drawn, and  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  were measured over 5 min. Immediately afterwards, a breath sample was obtained. These procedures were performed every 20 min until the end of exercise. After 60 min of cycling, a constant infusion of 20 μmol kg<sup>-1</sup> min<sup>-1</sup> sodium lactate labelled with 0.05% U-<sup>13</sup>C-lactate was started and continued until the end of exercise.

During the trial, water was available *ad libitum*. Upon cessation of exercise, volunteers were provided with a carbohydrate-rich snack. The first urine was then collected, the volume measured, and two aliquots of 10 ml were frozen at -20°C for later determination of urinary nitrogen.

On a separate occasion, a subgroup of participants ( $n = 5$ ), recruited from NOR ( $n = 2$ ) and HYP ( $n = 3$ ), underwent 180 min of exercise at the same relative intensity and in similar nutritional conditions without exogenous lactate infusion to assess the evolution of plasma lactate concentration during 180 min of continuous exercise. During this trial, blood samples were drawn at 0 and 40 min and then every 20 min for a total of 180 min. The results showed that without exogenous lactate infusion the plasma lactate concentration remained stable from 60 to 180 min (Fig. 2).

## POST training tests

On the last day of the recovery week, the POST training period began with assessment of  $\dot{V}_{O_{2max}}$  and MAP, as explained before ('*Exercise performance tests*'), although no familiarization TT was performed at the end of this session. Thereafter, the three other tests were performed

as explained for the baseline period, and the procedures of each test were identical. However, the workload of the lactate–glucose turnover assessment test was adapted to the measured MAP after training. A blood sample for determination of haematological variables was drawn at rest either before the  $\dot{V}_{O_{2max}}$  test or before the time trial.

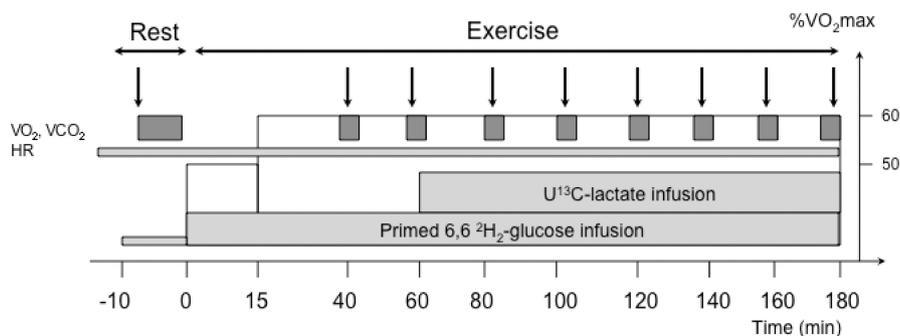
## Analytical procedures

Plasma was immediately separated from blood by centrifugation at 4°C for 10 min at 3600g and stored at -20°C. Lactate concentration was measured enzymatically using kits from Boehringer Mannheim (Mannheim, Germany). Plasma glucose concentration was measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA, USA). Colorimetric methods were used to assess plasma concentrations of FFAs (kit from Wako Chemicals, Freiburg, Germany). Commercial radioimmunoassay kits were used for the determination of plasma cortisol, insulin and glucagon. Plasma 6,6-<sup>2</sup>H<sub>2</sub>-glucose was measured by gas chromatography–mass spectrometry (Hewlett-Packard Instruments, Palo Alto, CA, USA) and expired air <sup>13</sup>CO<sub>2</sub> isotopic enrichment was measured by isotope-ratio mass spectrometry (SerCon Ltd, Crewe, UK). For details see Schneiter *et al.* (1999) and Chiolero *et al.* (2000).

Haematocrit (Hct, %) and haemoglobin mass (Hb, g l<sup>-1</sup>) were measured at inclusion and at the end of the fifth week of training. Haematocrit was measured using an impedance technique, and Hb measurement was performed by photometric dosage (Sysmex XE-2100, Sysmex, Hamburg, Germany).

## Calculations

**Lactate turnover.** Relative exogenous lactate oxidation was calculated from  $\dot{V}_{CO_2}$  and expired air <sup>13</sup>CO<sub>2</sub>



**Figure 1. Overview of the metabolic test**

Abbreviations: HR, heart rate recording;  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$ , measurement of oxygen uptake and carbon dioxide production, respectively. Vertical arrows represent blood and expired air sampling times. Exercise started at time 0 at an intensity eliciting 50%  $\dot{V}_{O_{2max}}$  and was increased after 15 min.

enrichment, adapted from Schneiter *et al.* (1995), as follows:

$$\text{Relative lactate oxidation (\% of infused dose)} = \left( \frac{100 \dot{V}_{\text{CO}_2} E_{\text{CO}_2}}{k E_{\text{lactate}}} \right) \left( \frac{1}{22.4 \times 3} \right) \left( \frac{1}{F} \right) \quad (2)$$

where  $\dot{V}_{\text{CO}_2}$  is the volume of expired  $\text{CO}_2$ ,  $E_{\text{CO}_2}$  the enrichment of expired  $\text{CO}_2$  expressed in atom percent excess,  $k$  the recovery of  $^{13}\text{C}$  in expired air,  $E_{\text{lactate}}$  the  $^{13}\text{C}$  isotopic enrichment (%) of the exogenous lactate infused, 22.4 is the volume of  $\text{CO}_2$  (ml) per millimole of  $\text{CO}_2$  produced, 3 is the number of millimoles of  $\text{CO}_2$  produced by oxidation of 1 mmole of lactate and  $F$  the infusion rate of exogenous lactate. Recovery of metabolic  $^{13}\text{CO}_2$  in breath was considered to be complete.

Lactate metabolic clearance rate (MCR) was calculated as the ratio between lactate infusion rate ( $\mu\text{mol kg}^{-1} \text{min}^{-1}$ ) and the increase of plasma lactate concentration ( $\mu\text{mol ml}^{-1}$ ) in response to lactate infusion, according to Revelly *et al.* (2005). Lactate MCR was calculated between 60 and 160 min because this method

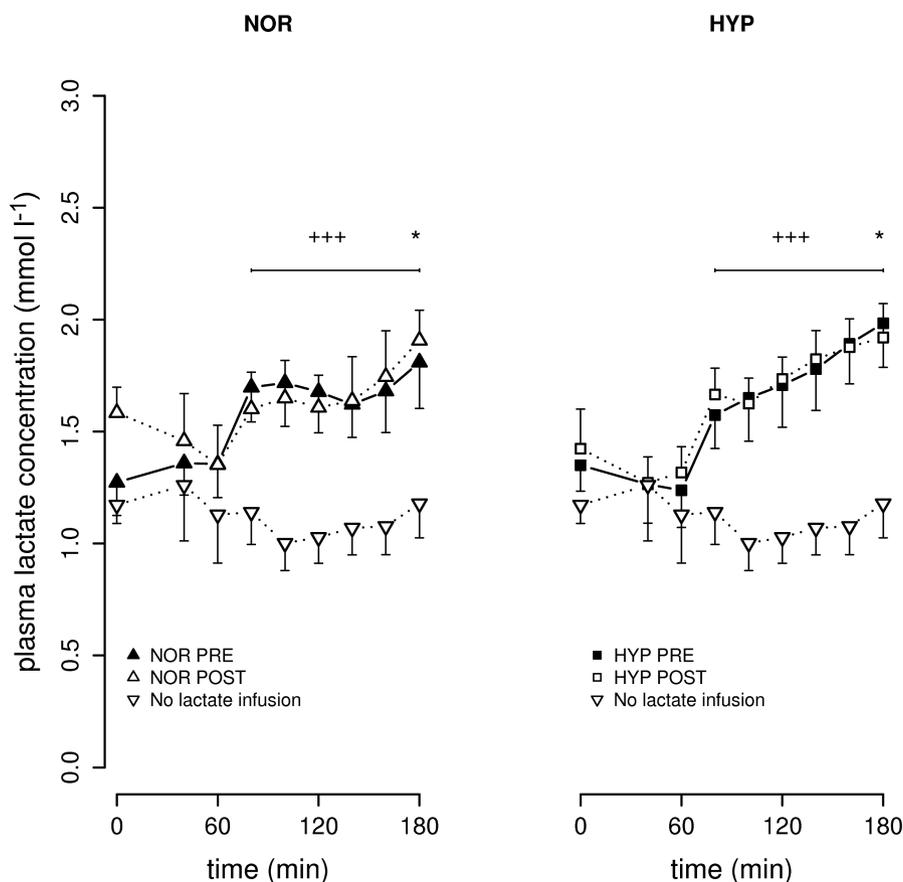
requires a steady state of plasma lactate concentration (Revelly *et al.* 2005), and a significant increase of plasma lactate was found at 180 min (Fig. 2).

Endogenous lactate production (ELP;  $\mu\text{mol kg}^{-1} \text{min}^{-1}$ ) was calculated as the product of baseline lactate concentration (first 60 min of exercise) and exogenous lactate clearance rate, assuming that endogenous lactate production was not altered by lactate infusion (Chiolero *et al.* 2000).

**Glucose turnover.** Glucose rates of appearance and disappearance, as well as metabolic clearance rate, were calculated with Steele's equations for non-steady-state conditions, as follows:

$$R_a G = \frac{F - V[(C_1 + C_2)/2][(E_1 - E_2)/(t_2 - t_1)]}{(E_2 + E_1)/2} \quad (3)$$

$$R_d G = R_a G - V \left( \frac{C_2 - C_1}{t_2 - t_1} \right) \quad (4)$$



**Figure 2. Evolution of plasma lactate concentration during the lactate turnover test before and after training in the NOR and HYP groups with and without exogenous lactate infusion**

+++ Significant effect of lactate infusion on plasma lactate concentration ( $P < 0.001$ ); \* significant effect of time ( $P < 0.05$ ). Values are shown as means  $\pm$  S.E.M.

$$\text{Glucose MCR (ml kg}^{-1}\text{min}^{-1}) = \frac{R_a G}{(C_2 - C_1)/2} \quad (5)$$

where  $R_a G$  and  $R_d G$  are the rate of appearance ( $R_a$ ) and disappearance ( $R_d$ ) of glucose ( $\mu\text{mol kg}^{-1}\text{min}^{-1}$ ), respectively,  $F$  is the infusion rate of 6,6- $^2\text{H}_2$ -glucose ( $\mu\text{mol kg}^{-1}\text{min}^{-1}$ ),  $V$  is the volume of distribution of glucose (180 ml  $\text{kg}^{-1}$ ),  $C_1$  and  $C_2$  are the glucose concentrations ( $\mu\text{mol ml}^{-1}$ ) at time 1 ( $t_1$ ) and 2 ( $t_2$ ), respectively, and  $E_1$  and  $E_2$  are the glucose enrichment in 6,6- $^2\text{H}_2$ -glucose (%) at  $t_1$  and  $t_2$ , respectively.

**Substrate oxidation.** Glucose and fat oxidation rates were calculated from respiratory gas exchanges (Fraysn, 1983), assuming that substrate oxidation rates were not altered by exogenous lactate infusion (Chiolero *et al.* 1993; Miller *et al.* 2002), as follows:

$$\text{Glucose oxidation (g min}^{-1}) = 4.55 \dot{V}_{\text{CO}_2} - 3.21 \dot{V}_{\text{O}_2} - 2.87N \quad (6)$$

$$\text{Fat oxidation (g min}^{-1}) = 1.67 \dot{V}_{\text{O}_2} - 1.67 \dot{V}_{\text{CO}_2} - 1.92N \quad (7)$$

where  $\dot{V}_{\text{O}_2}$  and  $\dot{V}_{\text{CO}_2}$  ( $\text{l min}^{-1}$ ) are oxygen consumption and carbon dioxide production, respectively, and  $N$  is urinary nitrogen excretion ( $\text{g min}^{-1}$ ).

**Cycling efficiency.** Submaximal  $\dot{V}_{\text{O}_2}$  values obtained during the incremental test were used to calculate submaximal cycling efficiency, according to Truijens *et al.* (2003). Gross cycling efficiency was calculated, between 40 and 80%  $\dot{V}_{\text{O}_{2\text{max}}}$ , as the ratio between mechanical power output generated by the cyclist and the metabolic power output ( $P_{\text{met}}$ ), calculated by:

$$P_{\text{met}}(\text{W}) = \left(\frac{1}{60}\right) \times 10^3 \times [4.1868 (4.047 + \text{RER})] \dot{V}_{\text{O}_2} \quad (8)$$

where 4.1868 is the conversion factor from kilocalories to kilojoules and (4.047 + RER) is the energy equivalent for 1 l of oxygen.

**Compliance to training.** Using power output or heart-rate data obtained during each supervised training session, an index of compliance to training was calculated as the time spent at or above 95% of the prescribed intensity divided by the prescribed amount of time at the given exercise intensity.

## Statistical analysis

Data distributions were first examined visually, and data showing a skewed distribution were log-transformed before statistical calculations.

The effect of treatment (i.e. training, hypoxia and their interaction) over time on metabolic variables was analysed using a three-way ANOVA with repeated measures. Performance outcomes and lactate kinetics were analysed using a two-way ANOVA with repeated measures. When necessary, Tukey's *post hoc* least significant difference pairwise multiple comparisons were performed. Paired Student's *t* test was used to determine differences in training volumes and intensity between NOR and HYP groups. Measures of association between variables were obtained using Spearman's rank correlation test.

All statistical computations were performed using 'R', an open-source statistical software package (R Development Core Team, 2008). Significance was considered at a  $P$  value  $< 0.05$ , and a tendency was considered by  $0.05 < P < 0.1$ . All data are expressed as means  $\pm$  S.E.M.

## Results

### Training in normoxia and hypoxia

The NOR and HYP groups completed  $97 \pm 2$  and  $95 \pm 2\%$  of the supervised training time, respectively ( $P = 0.6$ ). No difference in compliance to training was found between groups; the NOR and HYP groups were able to perform  $80 \pm 5$  and  $80 \pm 5\%$  of the high-intensity work bouts, respectively. Under supervision, NOR and HYP groups trained at an average power output of  $3.0 \pm 0.1$  and  $2.5 \pm 0.1 \text{ W kg}^{-1}$ , respectively ( $P < 0.01$ ). This represents an average intensity of  $57 \pm 1$  and  $61 \pm 1\%$  of MAP in the respective training conditions (i.e. normoxia and hypoxia;  $P < 0.05$ ). In addition to the 17.2 h of supervised training, the NOR and HYP groups trained outside the laboratory for  $25.6 \pm 2.8$  and  $27.3 \pm 2.6$  h, respectively ( $P = 0.7$ ).

### Haematological variables

Before and after training, Hct was  $42.1 \pm 0.7$  and  $42.0 \pm 0.6\%$ , respectively, for the NOR group and  $43.4 \pm 0.9$  and  $44.3 \pm 1.0\%$ , respectively, for the HYP group. Haemoglobin was  $146 \pm 3$  and  $145 \pm 2 \text{ g l}^{-1}$  before and after training, respectively, for the NOR group and  $151 \pm 3$  and  $151 \pm 4 \text{ g l}^{-1}$ , respectively, for the HYP group. No significant effect of training, hypoxia or their interaction was found for either haematological variable.

**Table 2. Selected maximal and submaximal performance indicators measured during the incremental tests performed in normoxia before (PRE) and after 4 weeks of training (POST) in normoxia (NOR) or hypoxia (HYP)**

		NOR		HYP		P value		
		PRE	POST	PRE	POST	Training	Hypoxia	T×H
$\dot{V}_{O_{2max}}$	(l min <sup>-1</sup> )	4.7 ± 0.1	5.1 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	0.03	0.96	0.12
$\dot{V}_{O_{2max}}$	(ml kg <sup>-1</sup> min <sup>-1</sup> )	69.1 ± 2.6	73.2 ± 3.8	68.0 ± 2.6	69.4 ± 2.6	0.02	0.47	0.14
MAP	(W)	361 ± 9	386 ± 8	355 ± 10	371 ± 13	<0.001	0.52	0.38
$\dot{V}_{E,max}$	(l min <sup>-1</sup> )	131 ± 5	132 ± 6	135 ± 6	142 ± 7	0.31	0.44	0.65
$P_{VT1}$	(W)	250 ± 7	282 ± 10	240 ± 12	265 ± 9	<0.001	0.33	0.67
$P_{VT2}$	(W)	299 ± 5	321 ± 7	295 ± 12	320 ± 13	<0.01	0.92	0.72
GE	(%)	24.9 ± 0.4	25.8 ± 0.8	24.9 ± 0.5	25.4 ± 0.5	0.21	0.93	0.74

Abbreviations:  $\dot{V}_{O_{2max}}$ , maximal oxygen uptake; MAP, maximal aerobic power;  $\dot{V}_{E,max}$ , highest ventilatory rate;  $P_{VT1}$  and  $P_{VT2}$ , power output at the 1st and 2nd ventilatory thresholds, respectively; GE, gross cycling efficiency; and T×H, training × hypoxia interaction. Values are shown as means ± s.e.m.

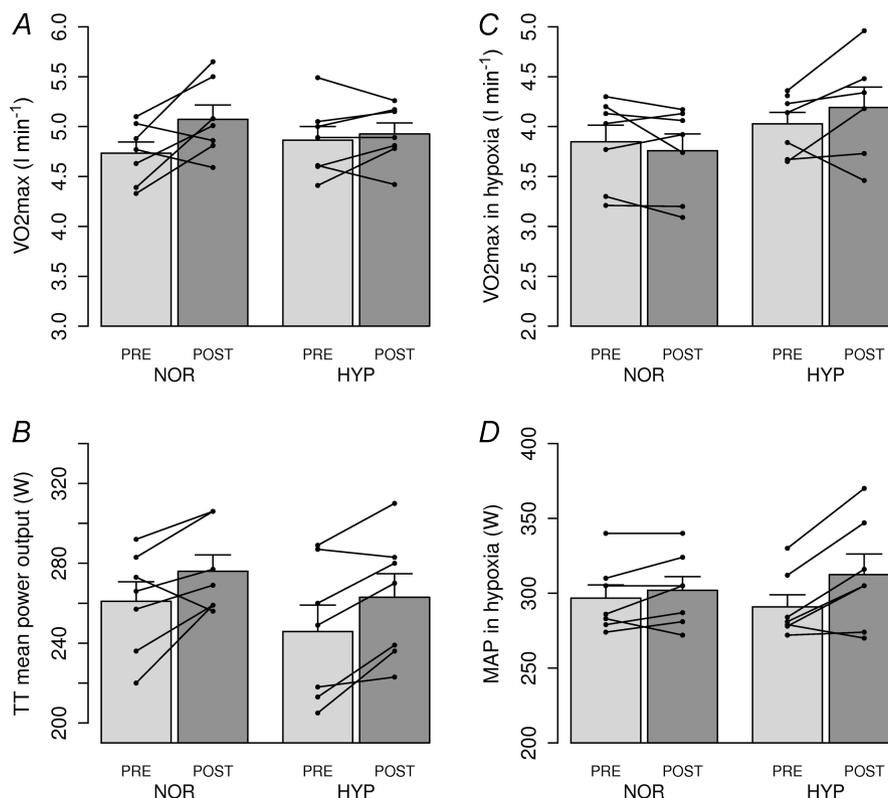
### Performance tests in normoxia

Individual  $\dot{V}_{O_{2max}}$  and TT performance data are shown in Fig. 3A and B. The overall results of the incremental test in normoxia are shown in Table 2.

After training,  $\dot{V}_{O_{2max}}$ , expressed in both absolute and relative terms, was significantly improved ( $P < 0.03$  and  $P < 0.02$ , respectively), and there was no difference between groups. The  $\dot{V}_{O_{2max}}$  increased by  $7.4 \pm 3.1\%$  in

the NOR group and  $1.4 \pm 1.7\%$  in the HYP group (n.s.). Similarly, MAP,  $P_{VT1}$  and  $P_{VT2}$  were significantly increased ( $P < 0.0001$ ,  $P < 0.004$  and  $P < 0.002$ , respectively), independently of group.

Time-trial performance was significantly improved after training ( $P < 0.01$ ; Fig. 3B). Mean power output sustained during the TT was increased from  $261 \pm 10$  to  $276 \pm 8$  W in the NOR group ( $+6.1 \pm 2.7\%$ ) and from  $246 \pm 13$  to  $263 \pm 12$  W in the HYP group ( $+7.4 \pm 2.1\%$ ).



**Figure 3. Absolute and individual evolution of aerobic performance in normoxia and hypoxia in the NOR and HYP groups, respectively**

A, evolution of  $\dot{V}_{O_{2max}}$  (l min<sup>-1</sup>). B, evolution of time-trial (TT) performance. C, evolution  $\dot{V}_{O_{2max}}$  in hypoxia. D, evolution of maximal aerobic power in hypoxia. Mean data are shown as means + s.e.m.

**Table 3. Selected maximal and submaximal performance indicators measured during the incremental tests performed in normobaric hypoxia (fractional inspired O<sub>2</sub> = 0.145) before (PRE) and after 4 weeks of training (POST) in normoxia (NOR) or hypoxia (HYP)**

		NOR		HYP		P value		
		PRE	POST	PRE	POST	Training	Hypoxia	T×H
$\dot{V}_{O_{2max}}$	(l min <sup>-1</sup> )	3.8 ± 0.2	3.8 ± 0.2	4.0 ± 0.1	4.2 ± 0.2	0.58	0.18	0.095
$\dot{V}_{O_{2max}}$	(ml kg <sup>-1</sup> min <sup>-1</sup> )	56.3 ± 3.2	54.8 ± 2.8	57.4 ± 1.9	58.3 ± 2.7	0.98	0.49	0.18
MAP	(W)	297 ± 9	302 ± 9	291 ± 8	312 ± 14	<0.01	0.87	0.06
$\dot{V}_{E,max}$	(l min <sup>-1</sup> )	115 ± 5	121 ± 6	119 ± 4	133 ± 6	<0.05	0.29	0.36
$P_{VT1}$	(W)	203 ± 11	210 ± 10	209 ± 8	219 ± 14	0.27	0.65	0.91
$P_{VT2}$	(W)	249 ± 8	255 ± 7	259 ± 6	251 ± 7	0.97	0.67	0.11
GE	(%)	22.9 ± 0.6	22.2 ± 0.4	23.1 ± 0.7	22.4 ± 0.5	0.42	0.87	0.77

Abbreviations are as in Table 2. Values are shown as means ± S.E.M.

**Table 4. Mean metabolic variables measured during prolonged exercise**

		NOR		HYP		P value		
		PRE	POST	PRE	POST	Training	Hypoxia	T×H
Lact. MCR	(ml min <sup>-1</sup> kg <sup>-1</sup> )	76.7 ± 16.1	73.1 ± 8.5	47.5 ± 6.8	52.9 ± 6.8	0.91	0.07	0.55
ELP	(μmol kg <sup>-1</sup> min <sup>-1</sup> )	103 ± 22	100 ± 18	59 ± 12	72 ± 12	0.69	0.09	0.55
Exo lact ox.	(% infused dose)	88 ± 12	107 ± 11	82 ± 3	90 ± 4	0.13	0.24	0.55
Gluc. ox.	(g min <sup>-1</sup> )	1.85 ± 0.18	2.2 ± 0.21	1.58 ± 0.21	2.03 ± 0.22	<0.001	0.38	0.28
Fat ox.	(g min <sup>-1</sup> )	0.58 ± 0.08	0.52 ± 0.08	0.52 ± 0.07	0.49 ± 0.09	<0.001	0.61	0.44

Abbreviations: Lact. MCR, lactate metabolic clearance rate; ELP, endogenous lactate production; Exo. lact. ox., exogenous lactate oxidation; Gluc. ox., glucose oxidation rate; Fat ox., fat oxidation rate; and T×H, training × hypoxia interaction. Values are shown as means ± S.E.M.

No interaction of training and hypoxia ( $P = 0.79$ ) was found on TT performance.

Changes in performance during the TT were significantly correlated with changes in power output at VT1 ( $\rho = 0.66$ ,  $P < 0.01$ ); however, no significant correlation between changes in TT performance and  $\dot{V}_{O_{2max}}$  or MAP was found ( $\rho = 0.15$ ,  $P = 0.6$  and  $\rho = 0.5$ ,  $P < 0.07$ , respectively).

### Performance tests in hypoxia

Group mean and individual evolution of  $\dot{V}_{O_{2max}}$  and MAP are shown in Fig. 3C and D, respectively. The  $\dot{V}_{O_{2max}}$  was increased by  $5.1 \pm 3.4\%$  in the HYP group and decreased by  $2.2 \pm 1.9\%$  in the NOR group ( $P = 0.12$ ). The MAP increased by  $1.8 \pm 1.3$  and  $7.2 \pm 2.3\%$  ( $P = 0.07$ ) in the NOR and HYP groups, respectively. Results of the incremental test performed in normobaric hypoxia are shown in Table 3. Briefly, training increased both MAP and maximal  $\dot{V}_E$  ( $P < 0.01$  and  $P < 0.05$ , respectively). No statistical interaction between training and hypoxia was found on any performance variable. However, HbO<sub>2</sub> saturation was slightly increased throughout the incremental test in the HYP group after training (training × hypoxia interaction,  $P = 0.05$ ).

### Metabolic test

Before training, subjects exercised for 180 min at an intensity corresponding to  $181 \pm 5$  and  $178 \pm 5$  W, also

corresponding to  $61.1 \pm 1.2$  and  $59.8 \pm 2.1\%$  of  $\dot{V}_{O_{2max}}$  for the NOR and HYP groups, respectively. After training, to match a similar relative exercise intensity, workload was increased to  $191 \pm 4$  and  $185 \pm 6$  W, which corresponded to  $60.5 \pm 2.8$  and  $60.5 \pm 1.6\%$   $\dot{V}_{O_{2max}}$  for the NOR and HYP groups, respectively. Consequently, absolute exercise intensity was higher POST training ( $P < 0.01$ ).

The evolution of plasma lactate concentration during prolonged exercise is shown in Fig. 2. Exogenous lactate infusion significantly increased lactate concentration ( $P < 0.001$ ). No effect of training or hypoxia was found on the evolution of plasma lactate concentration over time.

Evolution of lactate MCR, ELP and relative oxidation between PRE and POST is shown in Table 4. Briefly, no interaction between training and hypoxia was found on markers of lactate metabolism; however, NOR and HYP groups showed a tendency for differences in lactate MCR and ELP ( $P = 0.07$  and  $0.09$ , respectively).

Substrate oxidation rates were significantly different ( $P < 0.001$ ) after training (Table 4), owing to the higher energy expenditure associated with the higher absolute power output after training. Again, no interaction between training and hypoxia was found on these variables.

A significant interaction between training and hypoxia on the evolution of plasma glucose concentration ( $P < 0.05$ ) was found (Fig. 4). Similarly, insulin response was increased in the HYP group after training (interaction,  $P < 0.05$ ; Fig. 5). A significant interaction between hypoxia and training was also found on the insulin to glucagon ratio ( $P < 0.01$ ), with an increased insulin to glucagon

ratio after training being found in the HYP group. A significant effect of time on blood glucagon response ( $P < 0.001$ ) was found during prolonged exercise, but it was influenced neither by training nor by hypoxia. Similarly, plasma cortisol response was unchanged after treatment.

Glucose  $R_a$  and  $R_d$  rose significantly with time, from  $\sim 25 \mu\text{mol kg}^{-1} \text{min}^{-1}$  at the beginning to  $\sim 50 \mu\text{mol kg}^{-1} \text{min}^{-1}$  at the end of the exercise bout for both groups. A slight but significant decrease in glucose  $R_a$  was found after training in the NOR group ( $P = 0.05$ ). Glucose MCR was decreased after training in the HYP group (training  $\times$  hypoxia interaction,  $P < 0.01$ ; Fig. 6).

After training, plasma FFA concentration was lower ( $P < 0.001$ ) in the NOR and HYP groups, independent of treatment.

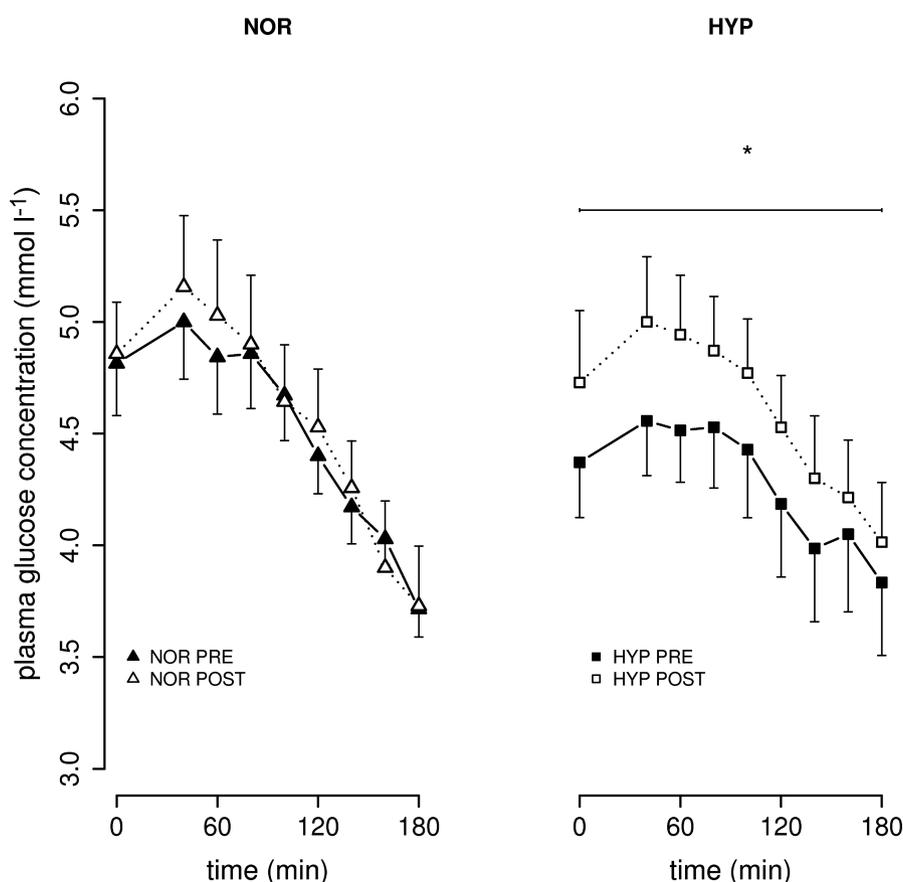
## Discussion

In contradiction to our hypothesis, our results show that hypoxia and training did not have synergistic effects

on endurance performance in normoxic conditions. Similarly, performance at simulated altitude was not significantly improved after 4 weeks of LLTH. Neither training nor hypoxia induced any significant changes in markers of lactate metabolism during exercise, but our results indicate that training in hypoxia slightly but significantly altered glucoregulation during prolonged exercise. This unexpected observation may have practical implications, because exercise training in hypoxia has recently been proposed as an efficient exercise prescription for patients with metabolic disorders (Haufe *et al.* 2008; Wiesner *et al.* 2009).

## Performance

After 4 weeks of high-intensity training, both groups exhibited similar increases in performance, indicating that hypoxia did not exert an additive effect on these performance outcomes. This finding is in line with previous work (Truijens *et al.* 2003; Ventura *et al.* 2003; Morton & Cable, 2005; Roels *et al.* 2005, 2007a) and



**Figure 4. Evolution of plasma glucose concentration during the lactate turnover test before and after training in the NOR and HYP groups**

\* Significant interaction between training and hypoxia on plasma glucose concentration ( $P < 0.05$ ). Values are shown as means  $\pm$  S.E.M.

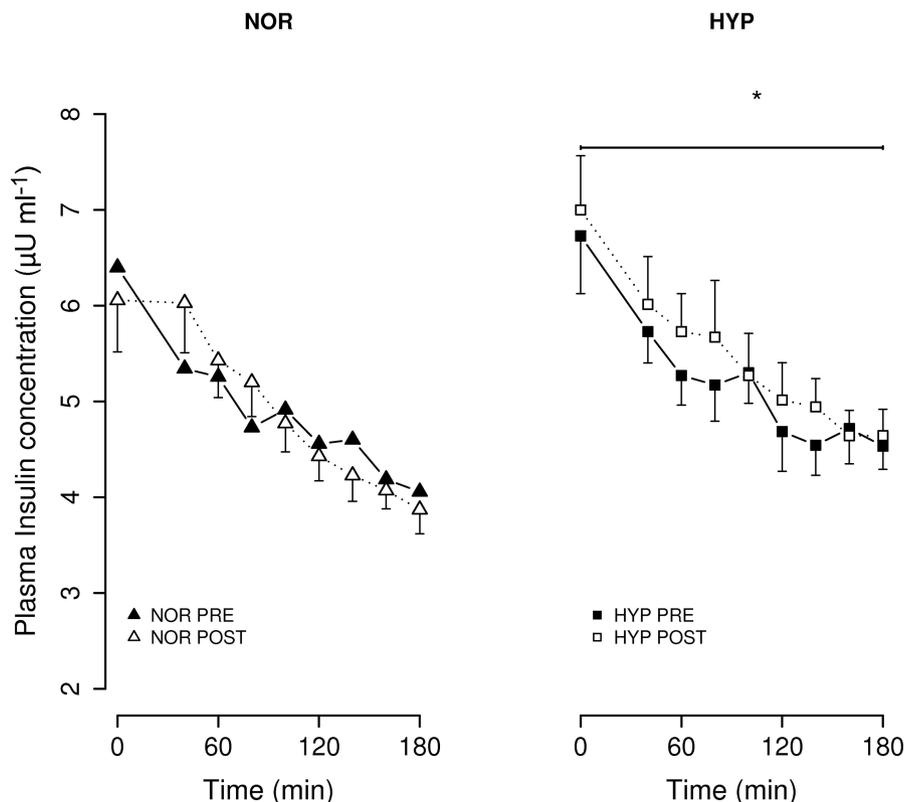
recent reviews (Levine, 2002; Hoppeler *et al.* 2008). In contrast, other studies reported significant benefits of a LLTH training regimen on endurance performance (Bailey *et al.* 2000; Meeuwsen *et al.* 2001; Dufour *et al.* 2006) or indicators of performance (Hendriksen & Meeuwsen, 2003; Dufour *et al.* 2006). According to a recent meta-analysis (Bonetti & Hopkins, 2009), exercise intensity is reported to be a major factor determining the magnitude of changes when training is performed in hypoxia, with moderate- to high-intensity training (i.e. close to VT2) more efficiently increasing performance (Dufour *et al.* 2006; Zoll *et al.* 2006) than higher exercise intensities (i.e. close to or equal to MAP; Roels *et al.* 2005, 2007a). To mimic training programmes used during general preparation in endurance cycling, the present study used a mixture of these training intensities, which overall may have been too high to improve performance, as suggested by Bonetti & Hopkins (2009). However, the improvements of performance reported in our study are consistent with those measured in previous training studies performed in normoxic conditions with endurance athletes (Stephens *et al.* 1999; Laursen *et al.* 2002, 2005; Faria *et al.* 2005). Specifically, these studies showed that 3–4 weeks of intense training by endurance cyclists produced

comparable enhancements of  $\dot{V}_{O_2 \max}$ , MAP and 40 km TT performance (Laursen *et al.* 2002, 2005).

One limitation of our study is that baseline training was not standardized; however, athletes were carefully recruited after at least one cycle (i.e. ~4 weeks) of basic endurance training either at the beginning or during the cycling season, which corresponds to a period long before the cyclists would have reached peak performance. Additionally, groups were studied in parallel, and training intensities were monitored throughout the study. Therefore, preceding training volume and intensity should not have confounded our results.

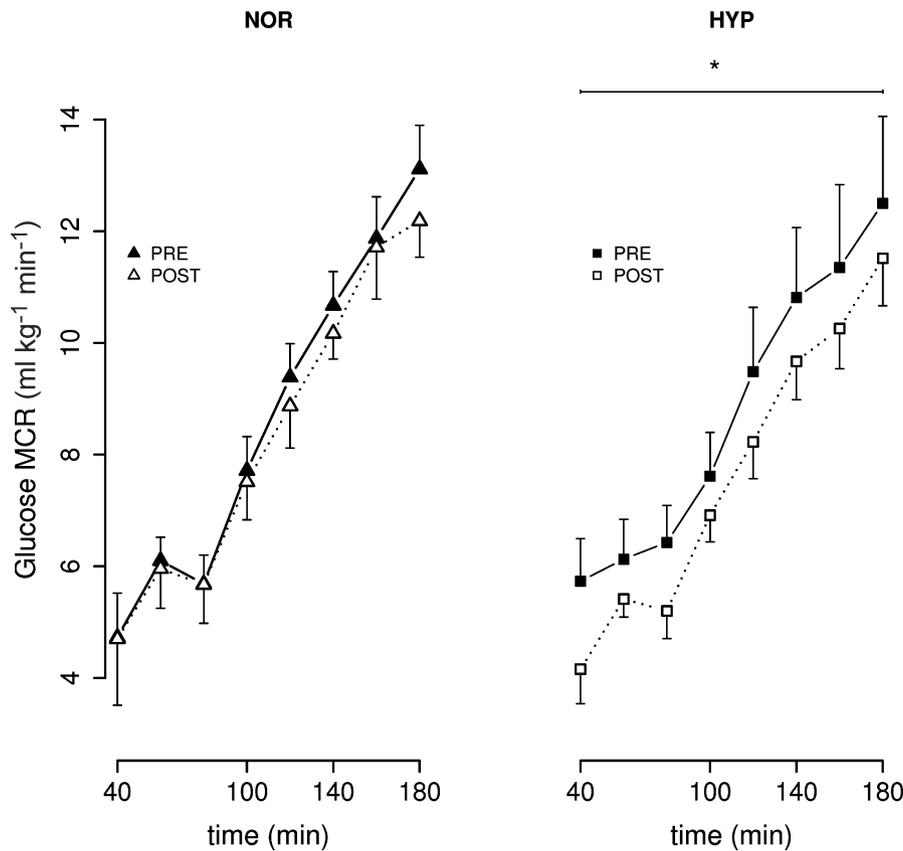
Altogether, these findings suggest that specificity of training, rather than the use of hypoxia during LLTH, is likely to have produced the performance enhancements measured in our study.

In hypoxic conditions, there was a trend (+7.2%) towards increased MAP in HYP (Table 3 and Fig. 3D), although it did not reach statistical significance, suggesting that performance in hypoxic conditions might be improved after LLTH. Previous studies reported enhanced performance at altitude without clear or significant increases in hypoxic MAP or performance indicators (Terrados *et al.* 1988; Vallier *et al.* 1996; Roels *et al.* 2007a).



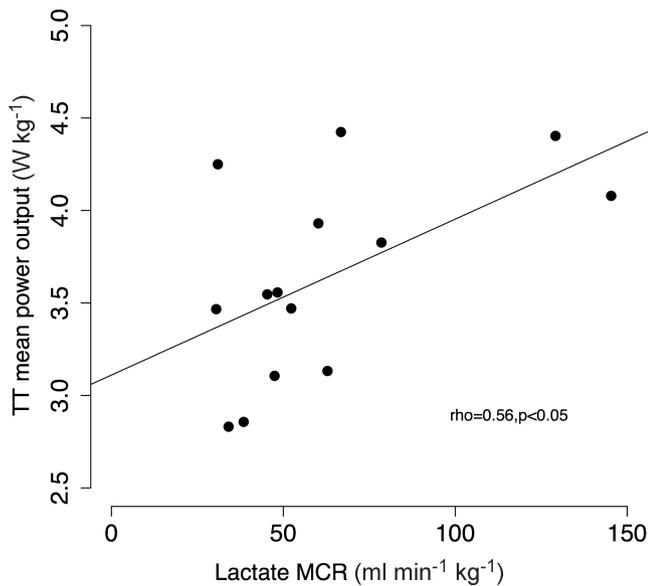
**Figure 5.** Evolution of plasma insulin concentration during the lactate turnover test before and after training in the NOR and HYP groups

\* Significant interaction between training and hypoxia on plasma insulin concentration ( $P < 0.05$ ). Values are shown as means  $\pm$  S.E.M.



**Figure 6. Evolution of glucose metabolic clearance rate (MCR) during the lactate turnover test before and after training in the NOR and HYP groups**

\* Significant interaction between training and hypoxia on glucose MCR ( $P < 0.05$ ). Values are shown as means  $\pm$  S.E.M.



**Figure 7. Correlation analysis between TT performance, expressed relative to body mass ( $W\ kg^{-1}$ ), and lactate metabolic clearance rate before the training intervention**

Spearman rank correlation:  $\rho = 0.56$  and  $P < 0.05$ .

Roels *et al.* (2007a) showed an improvement of MAP in hypoxic conditions concomitant with an increased ventilatory response, which led the authors to conclude that LLTH may be beneficial when preparing for competitions at altitude. Our results, however, provide no significant indication of an improved performance at altitude. Meeuwssen *et al.* (2001) showed a transient increase of Hb concentration after 10 days of LLTH. Furthermore, it is known that significant increases in aerobic performance at moderate altitude (i.e. 2500–3500 m) can be achieved through increasing Hb mass, hence, blood oxygen-carrying capacity (Robach *et al.* 2008), which may be achieved through sufficient exposure to hypoxic conditions (Levine & Stray-Gundersen, 2006) or erythropoietin administration (Robach *et al.* 2008). However, in the present study and in others (Vallier *et al.* 1996; Dufour *et al.* 2006; Roels *et al.* 2007a), no effect of LLTH was found on Hb mass.

It should also be mentioned that subjects were not blinded to treatment, which may have influenced the results of the incremental test performed in hypoxia and, to some extent, overall performance. Since training in hypoxia is associated with performance enhancements at sea level and at altitude, it is likely that the belief

of improved performance after altitude training might increase the athlete's motivation to complete the last stages of the incremental test. Unfortunately, estimating to what extent this motivational aspect impacts performance is difficult. In a carefully designed study, Clark *et al.* (2000) showed that the placebo effect of a nutritional treatment could account for up to 4% of the increases in performance and, therefore, concluded that 'researchers should recognize that observed enhancements of around 4% in power output could be due entirely to a placebo effect.' Our results show an increase of ~8% of MAP in the HYP group, although this increase was not significantly higher than the ~2% increase in the NOR group. Therefore, our study was probably underpowered to show any significant difference between groups. Considering the variability in our MAP measurements, a power calculation indicates that 17 subjects per group should have been included to show a significant increase in hypoxic MAP.

### Lactate clearance and endogenous production

Mean lactate MCR and ELP values reported in the present study are comparable to those reported by previous work in which lactate kinetics were measured by means of radioactive or stable isotopes tracers (Stanley *et al.* 1985; MacRae *et al.* 1992, 1995; Phillips *et al.* 1995; Bergman *et al.* 1999; Miller *et al.* 2002; Clark *et al.* 2004). These findings indicate that this pharmacokinetic model, previously used at rest (Revelly *et al.* 2005; Abdel-Sayed *et al.* 2008), can also be used during moderate prolonged exercise to determine lactate kinetics. However, substantial variability was observed between subjects, which has also been reported with isotope tracers (Phillips *et al.* 1995). Calculation of lactate MCR with this pharmacokinetic method relies on the exogenous lactate infusion rate and the absolute rise in plasma lactate concentration. Exogenous lactate infusion significantly increased plasma lactate concentration, but this increase was small in magnitude (Fig. 2). In addition, plasma lactate concentrations fluctuated with time during exercise, with or without the exogenous lactate infusion, thereby imparting variability in the calculated lactate kinetics. Better accuracy of the model may be achieved in forthcoming studies by increasing the rise in plasma lactate through greater exogenous lactate infusion. Our results also showed that, on average, 80–90% of exogenous labelled lactate was oxidized, thus confirming previous results showing that lactate was mainly cleared by and used for oxidative processes in trained individuals (MacRae *et al.* 1995; Van Hall *et al.* 2003). Based on our model, we were unable to measure any effect of training or hypoxia on either lactate MCR or lactate oxidation. We did, however, find a positive correlation between baseline TT performance and lactate MCR ( $\rho = 0.56$ ,  $P < 0.05$ ;

Fig. 7), which is consistent with previous studies showing that endurance performance is related to lactate MCR (Messonnier *et al.* 1997, 2002). Improved maintenance of intracellular pH through higher lactate efflux and removal rates has been proposed as a mechanism responsible for the increased endurance performance measured after training in previously untrained subjects (Messonnier *et al.* 2004; Zoll *et al.* 2006). Skeletal muscle lactate transport and dissipation may be one mechanism whereby endurance performance can be enhanced (Thomas *et al.* 2005; Messonnier *et al.* 2007). However, we were unable to find any association between changes in TT performance and lactate MCR ( $\rho = 0.23$ , n.s.). Thus, whatever mechanism is responsible for the relationship between lactate kinetics and performance, our results clearly indicate that LLTH failed to increase lactate MCR. Hence, it can be concluded that LLTH, compared with conventional training, does not improve muscle performance through changes in lactate shuttles, uptake or oxidation.

### Glucose metabolism

To our knowledge, the effect of LLTH training on glucose kinetics at the whole-body level in trained subjects has not been described. Results from earlier studies suggested improved glucose metabolism through increases in mRNA, protein contents or activity of key metabolic enzymes and transporters (Terrados *et al.* 1988; Zoll *et al.* 2006), as well as changes in mitochondrial respiration (Ponsot *et al.* 2006; Roels *et al.* 2007b) after LLTH training. These results led to the hypothesis that hypoxia and exercise may have synergistic effects on the metabolic profile of untrained subjects, which has been recently reported (Haufe *et al.* 2008).

In contrast to Haufe *et al.* (2008), our results of an interaction between exercise training and hypoxia on substrate kinetics indicate a possibly detrimental effect on whole-body glucose homeostasis, reflected by a systematic increase in plasma levels of glucose and insulin, a decrease in glucose MCR and an altered insulin to glucagon ratio. In healthy untrained volunteers, earlier studies showed either no effect of endurance training on glucose kinetics at a given relative intensity (Friedlander *et al.* 1997) or a decreased glucose MCR with a blunted decrease in insulin concentration (Coggan *et al.* 1990, 1995). It was suggested that decreased glucose MCR and blunted insulin response are metabolic adaptations to chronic endurance training (Coggan *et al.* 1990). Comparable plasma glucose levels before and after training and unchanged or decreased glucose  $R_a$  were also reported in these previous studies. The effects reported here in the HYP group are unlikely to be training-induced adaptations because our control group (i.e. NOR) did not exhibit such alterations of glucose kinetics but did show

a slightly decreased glucose  $R_a$ . Additionally, any effect of detraining due to the significantly reduced exercise intensity in the HYP group can be ruled out because both groups exhibited similar gains in exercise performance. Finally, any effect of acclimatization to altitude is also unlikely because substrate oxidation rates (Lundby & Van Hall, 2002) and glucose kinetics (Van Hall *et al.* 2002) are unchanged during exercise at a similar relative intensity after acclimatization to altitude. With regard to performance, a decrease in plasma glucose clearance may enhance endurance performance by delaying exercise-induced hypoglycaemia and may therefore represent a beneficial adaptation. However, this slight increase in glycaemia may also be due to some degree of muscle and/or hepatic insulin resistance, probably due to deleterious combined effects of high-intensity training and hypoxia. Altogether, it is likely that the altered glucose kinetics reported here do not reflect usual training adaptations but, rather, reflect a detrimental interaction between hypoxia and exercise training on glucose homeostasis.

Recently, it has been proposed that adapted LLTH training protocols are efficient therapeutic exercise interventions for populations with impaired glucose metabolism (Haufe *et al.* 2008; Wiesner *et al.* 2009). It should, however, be emphasized that in our study glucose kinetics were studied during exercise in a fasting state, whereas Haufe *et al.* (2008) reported the effect of LLTH on a glucose tolerance test at rest. Therefore, our observation of altered glucose kinetics after LLTH warrants further studies to assess its functional significance as well as the combined effects of various exercise intensities and hypoxia on glucose kinetics in resting and exercising conditions.

### Summary and perspectives

In summary, our data show that an LLTH training protocol produces no further enhancement of sea-level performance and lactate turnover than does a similar training programme performed in normoxia. Furthermore, gains in endurance performance in hypoxic conditions are unclear after LLTH. Our data indicate that in trained individuals the endurance performance is related to lactate kinetics, which may be assessed during exercise with exogenous lactate infusion and a pharmacokinetic model, although with limited sensitivity. The effects of modulating lactate kinetics on performance in already well-trained individuals remain to be explored.

Additionally, we report impaired blood glucose regulation after a LLTH training protocol, which suggests that in some circumstances, LLTH may lead to decreased insulin sensitivity and muscle glucose uptake. Therefore, these results indicate that combining exercise training and hypoxia may not be appropriate for patients with impaired

glucose metabolism. Further studies on the effects of hypoxic training on glucose metabolism at rest and during exercise are necessary.

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