Endurance training prevents negative effects of the hypoxia mimetic dimethyloxalylglycine on cardiac and skeletal muscle function

François Bertrand Favier, Florian A Britto, Benjamin Ponçon, Gwenaelle Begue, Béatrice Chabi, Cyril Reboul, Grégory Meyer, Guillaume Py

HIGHLIGHTED TOPIC | *Hypoxia 2015*

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Francois B. Favier,[1,2] Florian A. Britto,[1,2] Benjamin Ponçon,[3,4] Gwenaelle Begue,[1,2] Beatrice Chabi,[1,2] Cyril Reboul,[3,4] Gregory Meyer,[3,4] and Guillaume Py[1,2]

[1]*Institut National de la Recherche Agronomique, UMR 866 Dynamique Musculaire et Métabolisme, Montpellier, France;* [2]*Université de Montpellier, Montpellier, France;* [3]*Laboratoire de Pharm-écologie cardiovasculaire, Avignon, France; and* [4]*Université d'Avignon, Avignon, France*

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Favier FB, Britto FA, Ponçon B, Begue G, Chabi B, Reboul C, Meyer G, Py G. Endurance training prevents negative effects of the hypoxia mimetic dimethyloxalylglycine on cardiac and skeletal muscle function. *J Appl Physiol* 120: 455–463, 2016. First published December 17, 2015; doi:10.1152/japplphysiol.00171.2015.— Hypoxic preconditioning is a promising strategy to prevent hypoxia-induced damages to several tissues. This effect is related to prior stabilization of the hypoxia-inducible factor-1␣via inhibition of the prolyl-hydroxylases (PHDs), which are responsible for its degradation under normoxia. Although PHD inhibition has been shown to increase endurance performance in rodents, potential side effects of such a therapy have not been explored. Here, we investigated the effects of 1 wk of dimethyloxalylglycine (DMOG) treatment (150 mg/kg) on exercise capacity, as well as on cardiac and skeletal muscle function in sedentary and endurance-trained rats. DMOG improved maximal aerobic velocity and endurance in both sedentary and trained rats. This effect was associated with an increase in red blood cells without significant alteration of skeletal muscle contractile properties. In sedentary rats, DMOG treatment resulted in enhanced left ventricle (LV) weight together with impairment in diastolic function, LV relaxation, and pulse pressure. Moreover, DMOG decreased maximal oxygen uptake (state 3) of isolated mitochondria from skeletal muscle. Importantly, endurance training reversed the negative effects of DMOG treatment on cardiac function and restored maximal mitochondrial oxygen uptake to the level of sedentary placebo-treated rats. In conclusion, we provide here evidence that the PHD inhibitor DMOG has detrimental influence on myocardial and mitochondrial function in healthy rats. However, one may suppose that the deleterious influence of PHD inhibition would be potentiated in patients with already poor physical condition. Therefore, the present results prompt us to take into consideration the potential side effects of PHD inhibitors when administrated to patients.

2-oxoglutarate; exercise; heart; mitochondrial respiration; prolyl hydroxylase inhibitor

HYPOXIA IS A CONDITION ENCOUNTERED during ascent to high altitude or in pathologies such as heart failure or chronic obstructive pulmonary disease. Prolonged reduction in O_2 pressure leads to cellular adaptations to cope with the energetic challenge imposed by O_2 rarefaction. Hypoxia-related effects are mainly due to activation of the hypoxia-inducible factor (HIF)-1. Indeed, HIF-1 transcription factor triggers the expression of more than 100 genes notably involved in glycolysis, vascular remodeling, iron metabolism and erythropoiesis (40). HIF-1 is a heterodimer composed by a ␣-subunit that is constitutively expressed and a ␣subunit whose expression is O_2 dependent. Under normoxia, HIF-1␣ protein is quickly addressed for proteasomal degradation. This mechanism is triggered by prolyl-hydroxylases (PHDs) that require Fe^{2+}, 2-oxoglutarate and oxygen to hydroxylate proline residues on HIF-1␣(16, 17). Under hypoxia, PHD activity decreases due to cofactors (Fe^{2+}) and substrate depletion (O_2), leading to HIF-1␣ accumulation and dimerization with the ␣-subunit. Moreover, HIF-1␣ hydroxylation on asparagine residue via factor inhibiting HIF-1 represses its transcriptional activity under normoxia (37).

Therefore, inhibition of PHD has been proposed to mimic cellular adaptations to hypoxia, and PHD inhibitors (PHI) have been successfully used to increase expression of HIF-1 targets, notably erythropoietin (EPO) (15) or vascular endothelial growth factor (3). HIF-1␣hydroxylation can be inhibited by iron chelators, such as cobalt chloride (a widely used hypoxia mimetic) and ethyl-3,4-dihydroxybenzoate (EDHB) or 2-oxoglutarate antagonists like dimethyloxalylglycine (DMOG). Because severe hypoxia, as encountered during cardiac ischemia, can lead to apoptosis (24), PHI can be of particular interest in the prevention of hypoxic damages. Indeed, previous reports showed that PHI-mediated hypoxic preconditioning is a promising way to protect tissues from severe hypoxia during ischemic episodes (8, 31). Importantly, PHI administration a few hours after myocardial infarction is still efficient to reduce infarct size (46), and DMOG has beneficial effects in pathologies related to other tissues, such as bone (48) or retina (44). In addition to this protective action in pathological models, iron-dependent PHI have been shown to increase aerobic performance under normoxia (39) or severe hypoxia (18). In this context, the ergogenic effect of 2-oxoglutarate antagonists still needs to be explored.

Nevertheless, HIF-1-related effects of hypoxia can be detrimental for oxidative metabolism (6, 41). For example, hypoxia decreases mitochondrial function via reduction of mitochondrial enzymes activity, respiration rate, and content (10, 14, 36). This, in turn, will result in reduced muscle endurance and consequently higher muscle fatigability. In addition, hypoxia induces cardiac remodeling (34, 35), and prolonged HIF-1␣ stabilization causes cardiomyopathy with impairment of heart function in genetic models (4, 21, 29, 30). In just a few months,

Address for reprint requests and other correspondence: F. B. Favier, UMR Institut National de la Recherche Agronomique 866 DMEM, bât 22, 2 place Viala, F-34060 Montpellier, France (e-mail: ffavier@univ-montp1.fr).

three professional athletes have been tested positive to 2-oxoglutarate antagonist. Taken as a whole, these data stress the need to identify potential deleterious effects of PHI.

In this study, we determined whether 2-oxoglutarate antagonist DMOG enhances exercise endurance performance in rats. We also depict DMOG-induced adaptations on hematological parameters (polycythemia) and on skeletal, as well as cardiac muscle function to define the central and/or peripheral effects of DMOG on performance. Lastly, because of the potential side effects of PHI, we examined whether regular aerobic exercise optimizes PHI-mediated adaptations.

MATERIALS AND METHODS

Animals

Twelve-week-old male Wistar rats (Charles River, L'Arbresle, France) were used for all experiments except for EPO determination, for which 10-wk-old C57 BL/6J mice were obtained from our animal facility. Animals were housed under standard conditions with a 12:12-h light-dark cycle, and access to food and water ad libitum. All procedures were approved by the Ethics Committee of the Languedoc-Roussillon for animal experiment (project: CE-LR-11007).

Protocol

Forty rats were divided into four groups: sedentary control (SED CTRL), sedentary DMOG-administered (SED DMOG), trained control (TRAIN CTRL), or trained DMOG-administered (TRAIN DMOG; $n = 10$/group). Training consisted in treadmill running 5 days a week during 6 wk. The duration and the speed were progressively increased during the first week to reach 40 min and 25 m/min, respectively. The five next weeks were performed at 25 m/min for 30 min after a 10 min warmup. During the last week of the training, rats were intraperitoneally administrated with either 150 mg·day^{-1}·kg^{-1} body wt of DMOG in NaCl 0.9% (DMOG groups) or a similar volume of NaCl 0.9% (CTRL groups).

Hematologic Parameters

Blood sampling was performed on rats to measure total hemoglobin (Avoximeter 4000; A-VOX Systems, San Antonio, TX) and hematocrit (HAEMATOKRIT 210, Hettich, Tuttlingen, Germany) at the end of the protocol. Because of the kit compatibility, C57BL/6J mice received ip injection of DMOG or EDHB (100 and 200 mg/kg body wt) and were killed 8 h after administration for plasma EPO determination using a mouse EPO ELISA kit (R&D Systems, Minneapolis, MN). Previous study on EPO induction with PHI showed that EPO level was close to the maximal concentration 8 h after injection (15).

Running Performance

We determined the maximal aerobic velocity (MAV), a marker of skeletal muscle oxidative metabolism, for every rat during a treadmill running test, as previously described (34). Briefly, speed was set to 10 m/min for 3 min, after which it was increased by 4 m/min every 90 s until ↓ 80% of the expected MAV was reached. Then, the speed (26 and 34 m/min for sedentary and trained groups, respectively) was increased by 1 m/min every 60 s until exhaustion. We used a different schedule between sedentary and trained rats to keep exercise duration in a similar range (15 to 20 min) for all animals. The test took place 24 h after the last training session for TRAIN groups. Sedentary rats were acclimatized to the treadmill on the 3 days preceding the test (10 min at 15 m/min).

Aerobic endurance was assessed 24 h after MAV determination. Rats were asked to run until exhaustion at 85% of their MAV both in normoxia and moderate hypobaric hypoxia (simulated altitude of 3,000 m). Rats were randomly assigned to normoxic or hypoxic session, each test being separated from the preceding by 24 h.

Skeletal Muscle Contractile Properties

Twenty-four hours after the last time trial session, rats were anesthetized with pentobarbital sodium (100 mg/kg) and extensor digitorum longus (EDL) muscle was removed for determination of contractile properties. Briefly, the distal tendon of the muscle was tied to a fixed pin in the organ bath, while the proximal tendon was attached to the lever arm of a dual-mode servomotor (305-LR; Aurora Scientific, Aurora, ON, Canada). Muscles were stimulated along their entire length with platinum wire electrodes at their optimum muscle length (L0), i.e., the muscle length producing maximal twitch tension. All subsequent measurements were made at L0. Muscles were preincubated for 15 min in Krebs-Ringer buffer (containing in mM: 120 NaCl, 4.8 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, and 1.2 $MgSO_4$; pH 7.4) supplemented with 5 mM HEPES, 5 mM glucose and saturated with 95% O_2-5% CO_2 gas mixture, maintained at 30°C. A computer generated the signal for electrical stimulation and force/length control of the levers during the contractions through commercial software (DMC v. 4.1.6, Aurora Scientific). The tension-frequency response was then determined (701B Stimulator, Aurora Scientific) using a stimulation trains of 500 ms, with pulse duration of 0.5 ms, at frequencies of 25, 75, 100, 150, and 200 Hz. Stimulus trains were separated by a 1-min interval. The maximum isometric tetanic tension (P0) was then determined. Five minutes after the tension-frequency determination, the resistance to fatigue was evaluated using repeated contractions (200-Hz trains of 120 ms, evoked twice per second) until the muscle lost 50% of its initial force. The muscle fatigue index (Tlim) was defined as the time taken to reach 50% of P0. After all measurements, muscles were removed from the bath, trimmed of the connective tissue, blotted dry, and weighed.

Skeletal Muscle Mitochondrial Respiration

Gastrocnemius muscles were quickly excised and immediately placed into ice-cold buffer (100 mM KCl, 5 mM $MgSO_4$, 5 mM EDTA, 50 mM Tris·HCl, pH 7.4). Mitochondria were fractionated by differential centrifugation, as described previously (20). Briefly, muscles were freed of connective tissues, minced, homogenized with an Ultra-turax homogenizer, and treated with subtilisin A (0.1 mg/g wet muscle). Mitochondria were separated by centrifugation at 8000 *g*, then at 800 *g*. Finally, mitochondria were pelleted from the supernatant at 9000 *g*. Mitochondria were resuspended in 100 mM KCl and 10 mM MOPS, at pH 7.4. Mitochondrial protein content was determined using the Bradford assay.

Mitochondria oxygen consumption was measured using the high-resolution Oxygraph-2k (OROBOROS Instruments, Innsbruck, Austria). Mitochondria were incubated in two sealed thermostated chambers (37°C) containing 2 ml of MIRO5 respiration medium (0.5 mM EGTA, 3 mM $MgCl_2 \cdot 6H_2O$, 65 mM KCl, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, and 1 g/l BSA, pH 7.1). Resting rate (state 4) was evaluated in the presence of 2.5 mM malate, 5 mM glutamate; ADP-stimulated rate (state 3) was determined after addition of 0.5 mM ADP. Data acquisition and analysis were performed using Oxygraph-2k-DatLab software version 4.3. The respiratory control ratio (RCR) was set as the ratio of oxygen consumption at state 3 over oxygen consumption at state 4.

Skeletal Muscle Biochemical Analyses

LDH isoforms. LDH isoenzymes from plantaris muscle were separated, as previously described (28). Briefly, ↓ 20mg of muscle was homogenized in 210 mM sucrose, 2 mM EGTA, 5 mM EDTA, 40 mM NaCl, 30 mM HEPES, pH 7.4 with protease inhibitors, and samples were centrifuged at 10,000 *g* for 5 min. Twenty-five micrograms of protein from the supernatant were separated by agarose gel

electrophoresis, and gels were stained with a solution containing 208 mM Li-L-lactate, 5.6 mM NAD, 2.4 mM p-nitro blue tetrazolium chloride, and 0.33 mM phenazine methosulfate. The reaction was blocked in a 10% acetic acid and 5% methanol solution. Lastly, LDH isoenzymes bands were scanned, and optical density was quantified.

Glycogen content. Muscle glycogen content was measured as previously described (25). Briefly, 30-50 mg of plantaris muscle was dissolved in 30% KOH saturated with Na_2SO_4 at 100°C for 20 min. Glycogen was precipitated by the addition of 1.2 vol of 95% ethanol, and the sample was centrifuged at 840 g (20°C) for 20 min. The glycogen precipitate was dissolved in 3 ml of distilled water. One milliliter of 5% phenol solution and 5 ml of H_2SO_4 were added to 200 l of the obtained glycogen solution, which was then incubated at room temperature for 10 min. Glycogen concentration was determined spectrophotometically at 490 nm.

HIF-1 immunoblotting. HIF-1 accumulation was measured in the nuclear fraction of gastrocnemius muscle 5 h after administration of DMOG (150 mg/kg). Muscle was homogenized in STM buffer [250 mM sucrose, 50 mM Tris·HCl (pH 7.4), 5 mM $MgCl_2$, protease/phosphatase inhibitor cocktail], and lysate was centrifuged at 800 *g* to separate nuclear fraction (pellet) from others (supernatant). Nuclear fraction was washed one time in STM buffer and the pellet was resuspended in NET buffer [20 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 0.5 M NaCl, 0.2 mM EDTA, 20% glycerol, 1% Triton X100, protease/phosphatase inhibitor cocktail]. Protein concentrations were determined using the BCA assay (Interchim, Montluçon, France). Fifty micrograms of protein were subjected to SDS-PAGE, as previously described (7). Membranes were probed with primary antibody against HIF-1 (NB100-123; Novus Biologicals, Littleton, CO).

mRNA expression analysis. mRNA extraction and gene expression analysis were performed, as previously described (7). Briefly, total RNA was extracted from the plantaris muscle using the RNeasy fibrous tissue kit (Qiagen, Venlo, The Netherlands). cDNA was then synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) from 1 g of total mRNA. Quantitative PCR was performed using KAPA 2 SYBR Green Master Mix on a Miniopticon thermocycler (Bio-Rad, Hercules, CA). Cycling conditions were one cycle at 98°C for 30 s followed by 40 cycles at 95°C for 1 s and 60°C for 15 s. Fusion index was measured by increments of 0.5°C every 5 s (starting at 65°C, finishing at 95°C). Each sample was run in duplicate. Sequences of the mouse forward and reverse primers are listed in Table 1. Results were expressed using the comparative cycle threshold with ribosomal protein S9 as the control gene.

Cardiac Morphology and Function

Doppler echocardiography. Cardiac morphology and function were evaluated via a noninvasive Doppler-echocardiography method, as previously described (35). Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (120 mg/kg ip). A two-dimensional view of the left ventricle (LV) was obtained at the level of the papillary muscles with a Mylab 30 ultrasound apparatus (ESAOTE, Italy). M-mode tracings were recorded through the anterior and posterior walls. End-diastolic anterior and posterior wall thicknesses (AWTd and PWTd, respectively) and LV internal end-diastolic (LVEDd) and systolic (LVEDs) diameters were measured from at least three consecutive cardiac cycles on the M-mode tracings. Diastolic relative wall thickness was calculated as [(AWTd + PWTd)/LVEDd] × 100 and was used as an index of LV geometry. Pulsed-wave Doppler spectra of mitral inflow were recorded from an apical four-chamber view. Peak velocities of early diastolic rapid inflow (peak E), atrial contraction filling (peak A), as well as their ratio (E/A), were recorded and served as indexes of diastolic function. Pulsed-wave Doppler spectra of the RV outflow were also recorded from a parasternal view of the pulmonary artery obtained at the level of the aorta. Measurement of the pulmonary peak flow velocity (Vpulm) was recorded as a negative index of pulmonary artery pressure. For all variables, measurements represent the mean of at least three consecutive cardiac cycles.

LV pressure. Systolic and diastolic LV pressures were performed on intact closed-chest anesthetized rats, via intraventricular catheterization (Mikro-Tip Pressure Catheter, Millar Instruments, Houston, TX), as previously described (1). Pulse pressure is the difference between the systolic and diastolic pressure. This hemodynamic evaluation was conducted under basal conditions and during -adrenergic challenging obtained by isoproterenol infusion (1 mg/kg ip).

Table 1. *Primers used for real-time quantitative PCR*

Gene	Forward Primer (5′→3′)	Reverse Primer (5′→3′)
HKII	GTTTCAAAGCGGTCGAACTGA	TTGGTCAACCTTCTGCACTT
NRF1	TTATTCTGCTGTGGCTGATG	GCTCTGATGCTTGCGTCGTCT
PFK	TCAGTGAGACAAGAACCCGT	CGTTTTTGTCGATTGCACCC
PGC-1	ATGTGTCGCCTTCTTGCTCT	ATCTACTGCCTGGGGACCTT
RPS9	GAAGCTGGGTTTGTCGCAAA	CGGAGCCCATACTCTCCAAT
Tfam	GCTAAACACCCAGATGCAAA	CGAGGTCTTTTTGGTTTTCC

HKII, hexokinase II; NRF1, nuclear respiratory factor 1; PFK, phosphofructokinase; PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1-; RPS9, ribosomal protein S9; Tfam, transcription factor A mitochondrial.

Statistical Analysis

EPO concentration was analyzed using one-way ANOVA to determine the effect of PHI dose (0, 100, or 200 mg/kg). Endurance performance was analyzed using three-way ANOVA to determine the main statistical effect of treatment, training, and ambience (normoxia vs. hypoxia), and interactions between these factors. All others parameters were analyzed using a two-way ANOVA to determine the main statistical effect of treatment (DMOG vs. CTRL) and training (sedentary vs. trained) and interactions between these factors. Fisher's least significant difference post hoc analysis was used to determine differences between groups when ANOVA was significant. Statistical significance was set at $P < 0.05$. Data are expressed as means SE.

RESULTS

HIF-1 Stabilization and Hematological Parameters

We first ensured that DMOG administration induced HIF-1 stabilization in skeletal muscle tissue. Fig. 1*A* shows HIF-1 accumulation in the nuclear fraction of gastrocnemius muscle 5 h after a single injection of DMOG. We next assessed whether this accumulation was associated with an increase in HIF-1 transcriptional activity by measuring plasma EPO, a direct target of HIF-1. Acute DMOG administration resulted in a robust and dose-dependent increase in EPO concentration 8 h after injection (12- and 24-fold with 100 and 200 mg/kg, respectively; Fig. 1*B*). We next checked whether repeated DMOG administration led to erythropoiesis in sedentary and trained rats. Consistently, 7-day treatment with 150 mg/kg of DMOG caused a significant increase in hematocrit and blood hemoglobin in both sedentary and trained rats (Fig. 1, *C* and *D*). In sharp contrast, the iron-dependent PHI EDHB induced a small increase in EPO concentration only with 200 mg/kg and did not alter hematocrit levels after a 7-day treatment (not shown). Thus, we focused on DMOG treatment.

Exercise Performance and Muscle Contractile Properties

Training significantly increased maximal aerobic velocity (MAV) in both DMOG and CTRL-treated rats (training effect

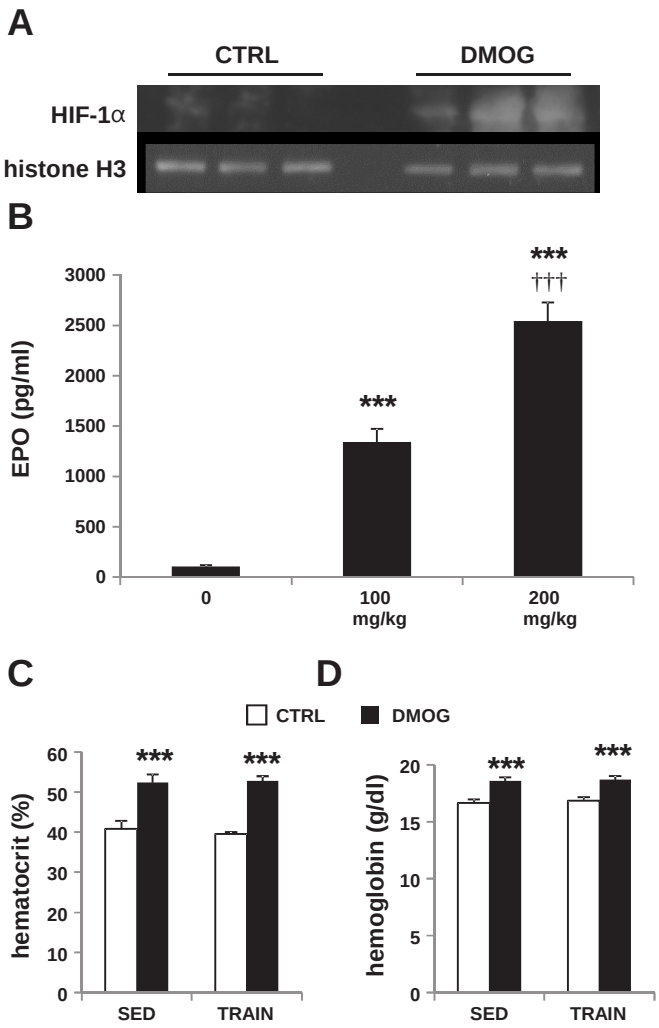

Fig. 1. Effects of acute and chronic dimethyloxalylglycine (DMOG) administration on HIF-1 stabilization and hematological parameters. *A*: HIF-1 ␣ protein content in the nuclear fraction of rat gastrocnemius muscle 5 h after DMOG (150 mg/kg) or vehicle injection (CTRL). *B*: plasma erythropoietin (EPO) concentration in mice receiving 100 or 200 mg/kg of DMOG (n = 3 or 4/group). ***P ↕ 0.001 vs. control, and †††P ↕ 0.001 vs. 100 mg/kg. *C* and *D*: hematocrit and hemoglobin concentration in sedentary (SED) or trained (TRAIN) rats subjected to 1 wk DMOG (DMOG) or NaCl 0.9% (CTRL) treatment (n = 8–10/group). ***P ↕ 0.001 vs. corresponding CTRL.

P ↕ 0.001; Fig. 2*A*). In addition, 150 mg/kg of DMOG during 7 days improved MAV (DMOG effect P ↕ 0.001). Interestingly, training and DMOG had synergic effects since DMOG had greater effects on MAV in trained rats (training × DMOG interaction P ↕ 0.001). We next determined aerobic endurance in normoxia and mild hypoxia during running test until exhaustion at 85% of normoxic MAV (Fig. 2*B*). Endurance was positively affected by treatment (DMOG vs. NaCl 0.9%; P = 0.03) and training (P = 0.025). In contrast, ambience had a negative effect on performance (hypoxia vs. normoxia; P = 0.005). There was no significant interaction between these three factors. Finally, we measured contractile properties of isolated EDL muscles. Neither maximal force nor resistance to fatigue during intermittent contractions was altered by either training or DMOG administration (Fig. 2*C*).

Cardiac Morphology

DMOG treatment significantly increased heart (P = 0.004) and LV weights (P = 0.007) in SED rats (Fig. 3). In contrast, no effect of DMOG on these variables was observed in trained animals. Endurance training had no effect on either heart or LV mass. Similarly, RV weight was not altered by either DMOG

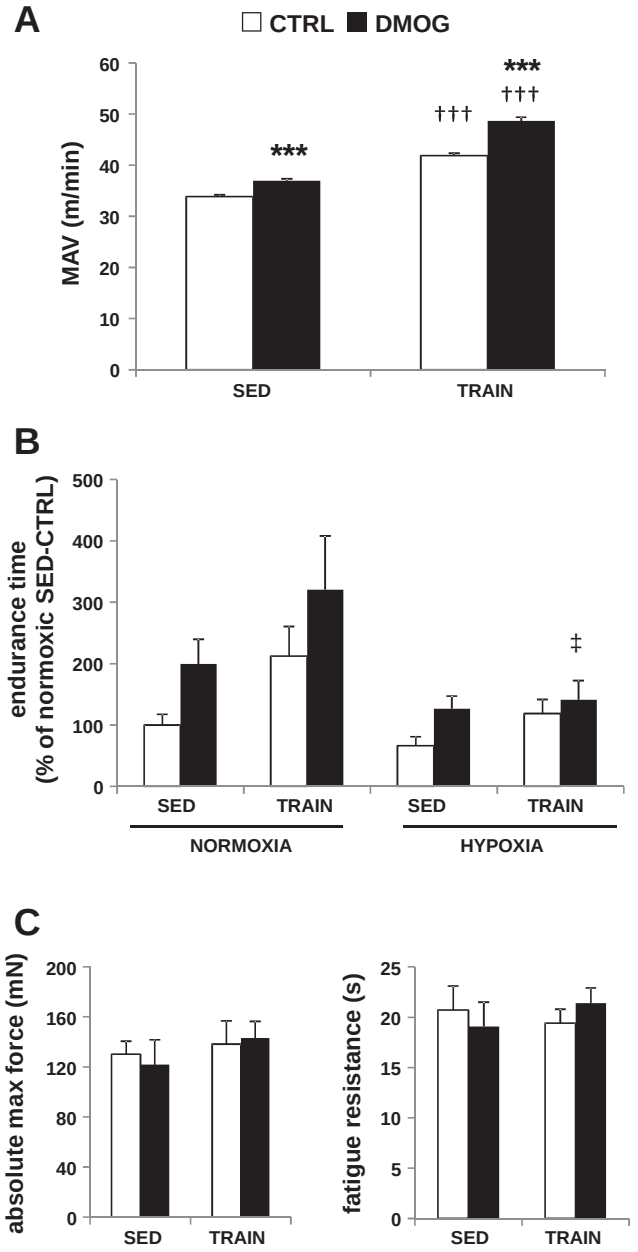

Fig. 2. Effect of DMOG administration on exercise performance and muscle contractile properties. *A*: maximal aerobic velocity of sedentary (SED) or trained (TRAIN) rats subjected to 1 wk of DMOG or NaCl 0.9% (CTRL) treatment (n = 8 or 9/group). *B*: endurance time in sedentary (SED) or trained rats subjected to 1 wk of DMOG (DMOG) or NaCl 0.9% (CTRL) treatment. Rats were subjected to treadmill running until exhaustion at 85% of their normoxic MAV in both normoxic and hypoxic environment. *C*: maximal force and resistance to fatigue of isolated EDL in sedentary (SED) or trained rats subjected to 1 wk DMOG or NaCl 0.9% (CTRL) treatment. ***P ↕ 0.001 vs. corresponding CTRL. †††P ↕ 0.001 vs. corresponding SED group. ‡P ↕ 0.05 vs. corresponding normoxic group.

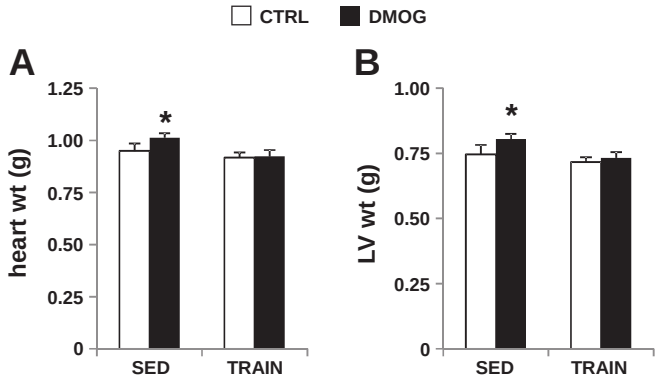

Fig. 3. Effects of DMOG treatment on heart and left ventricular mass. Heart (*A*) and left ventricle (LV) (*B*) weights in SED or trained rats subjected to 1 wk of DMOG or NaCl 0.9% (CTRL) treatment. *P ↕ 0.05 vs. corresponding CTRL.

treatment or endurance training (not shown). Expression of heart and LV weight relative to body weight showed a positive effect of both DMOG and training (not shown). However, the training effect resulted from a lower body weight (⩾10%) in exercised rats. Lastly, echocardiographic exploration revealed no significant alteration of LV morphology whatever the group considered (Table 2).

Cardiac Function

We assessed cardiac function under basal and ␣-adrenergic stress conditions. On the one hand, we founded no effect of DMOG treatment and/or endurance training on pulmonary peak flow velocity (not shown), LV pulse pressure, and maximal and minimal LV derivative pressures under basal conditions (Table 3). However, the E/A ratio, an index of diastolic function, was significantly impaired by DMOG treatment (P // 0.04; Fig. 4*A*). Importantly, post hoc analysis revealed significant differences between DMOG-treated and CTRL animals in SED (P // 0.01) but not in trained groups. On the other hand, DMOG had greater effects under ␣-adrenergic stimulation. Indeed, LV pulse pressure was decreased by DMOG administration in sedentary rats (P // 0.04; Fig. 4*B*). As for the E/A ratio, the negative effect of DMOG on pulse pressure was prevented by chronic exercise (significant differences between DMOG-treated and CTRL in SED but not trained rats). Similarly, the minimum derivative of LV pressure over time, an index of myocardial relaxation, was impaired by DMOG administration in SED but not in trained rats (P // 0.03; Fig. 4*C*). However, maximum derivative of LV pressure over time was not altered by either DMOG or training (Fig. 4*D*).

Muscle Metabolism

Oxidative capacity of muscle cells is tightly coupled to the mitochondrial activity. Respiration experiments on isolated mitochondria from gastrocnemius muscles revealed opposite effects of endurance training and DMOG administration (Fig. 5, *A* and *B*). Indeed, training had a main positive effect on ADP-stimulated maximal oxygen uptake (state 3; P // 0.002) and on the respiratory control ratio (RCR // state 3/state 4; P ↕ 0.001), while it tended to decrease the basal oxygen uptake (state 4; P // 0.07). On the contrary, DMOG had a main negative influence on state 3 (P // 0.004), whereas it increased state 4 (P ↕ 0.001). DMOG administration, thus, led to a decrease in RCR (P ↕ 0.001). There was no significant interaction between training and DMOG treatment. DMOG did not alter gene expression of PGC-1 ␣, NRF, and Tfam (Fig. 5*C*), three transcription factors involved in mitochondrial biogenesis, while training tended to increase PGC-1 ␣ mRNA level (P // 0.08). Besides, glycolytic metabolism is dependent on glycogen stores, as well as on the distribution of LDH isoforms, which promote either lactate production or oxidation. Glycogen content of plantaris muscle was not altered between the four groups (Fig. 6*A*). Similarly, we did not record any effect of training on LDH isoforms distribution (Fig. 6*B*). However, DMOG increased the activity of the LDH-4 (HM3) isoenzyme (main effect: P // 0.05), although post hoc analysis revealed no differences between groups. Lastly, we measured gene expression of two glycolytic enzymes upregulated by HIF-1. Again, hexokinase II and phosphofructokinase mRNA level was not altered by either DMOG or training (Fig. 6*C*).

DISCUSSION

Skeletal and cardiac muscles are metabolically active tissues that are highly dependent on oxygen availability. Therefore, a reduction in O_2 availability will induce phenotypic modifications of muscle cells. HIF-1 is essential for hypoxia acclimatization, as it regulates 89% of genes induced after exposure of mouse embryonic fibroblasts to 1% O_2 (12). Hypoxia adaptations can be mimicked by HIF-1 ␣ stabilization through PHD inhibition. In this study we investigated the effects of DMOG, a 2-oxoglutarate antagonist PHI, on cardiac and skeletal muscle function. We previously showed that 5 wk of moderate hypoxia (equivalent to 2,800 m) reduced LV filling (35). Here, only 1 wk of DMOG administration, which increased nuclear

Table 2. *Effects of dimethyloxalylglycine treatment on left ventricular morphology in sedentary and trained rats*

	SED		Trained	
	CTRL	DMOG	CTRL	DMOG
LVEDs, mm	0.312 ± 0.02	0.313 ± 0.023	0.304 ± 0.02	0.323 ± 0.021
LVEDd, mm	0.617 ± 0.016	0.63 ± 0.013	0.636 ± 0.009	0.64 ± 0.017
AWTd, mm	0.165 ± 0.006	0.175 ± 0.006	0.159 ± 0.008	0.15 ± 0.007
PWTd, mm	0.145 ± 0.006	0.16 ± 0.007	0.14 ± 0.005	0.15 ± 0.006
RWT, %	48.56 ± 1.76	53.29 ± 1.86	47.14 ± 2.33	47.01 ± 2.03

LVEDs, left ventricular end-systolic diameter; LVEDd, left ventricular end-diastolic diameter; AWTd, end-diastolic anterior wall thickness; PWTd, end-diastolic posterior wall thickness; RWT, relative wall thickness in sedentary (SED) or trained rats subjected to 1-wk dimethyloxalylglycine (DMOG) or NaCl 0.9% (CTRL) treatment (n // 8–10/group).

Table 3. *Effects of DMOG treatment on cardiac function at baseline in sedentary and trained rats*

	SED		Trained	
	CTRL	DMOG	CTRL	DMOG
LV pulse pressure, mmHg	135.3 ± 7.2	125.6 ± 4.4	123.4 ± 10.4	117.6 ± 4.9
dP/d*t* min mmHg/s	−6386 ± 454	−5920 ± 282	−5780 ± 540	−5413 ± 291
dP/d*t* max, mmHg/s	6806 ± 404	6450 ± 293	5937 ± 545	5791 ± 271

LV, left ventricle; dP/d*t* min and max, minimum and maximum derivative of left ventricle pressure in sedentary (SED) or trained rats subjected to 1 wk DMOG or NaCl 0.9% (CTRL) treatment (n = 8–10/group).

HIF-1α protein content in skeletal muscle, resulted in significant decrement in heart function, as attested by a decrease in LV filling rate (E/A), pulse pressure, and relaxation (dP/d*t* min). These data illustrate an alteration in diastolic function, which is potentiated under stress conditions, together with a slight impairment in systolic function. Interestingly, several studies reported that long term HIF-1α stabilization via genetic manipulation caused cardiomyopathy with LV dysfunction (4, 21, 29, 30). Bekeredjian et al. (4) notably observed that HIF-1α overexpression induced a decrease in the sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA)-2a together with a reduction in calcium reuptake. These mechanisms could explain the decrease in myocardial relaxation observed after HIF-1α stabilization in a previous (21) and the present study. We observed that DMOG administration also caused an increase in heart and LV weight. Again, this phenomenon has been reported in models with genetic HIF-1α stabilization (4, 21). Altogether, this strongly suggests that 1 wk of DMOG treatment initiates HIF-1-dependent cardiac remodeling that would be deleterious for cardiac function if administration was prolonged.

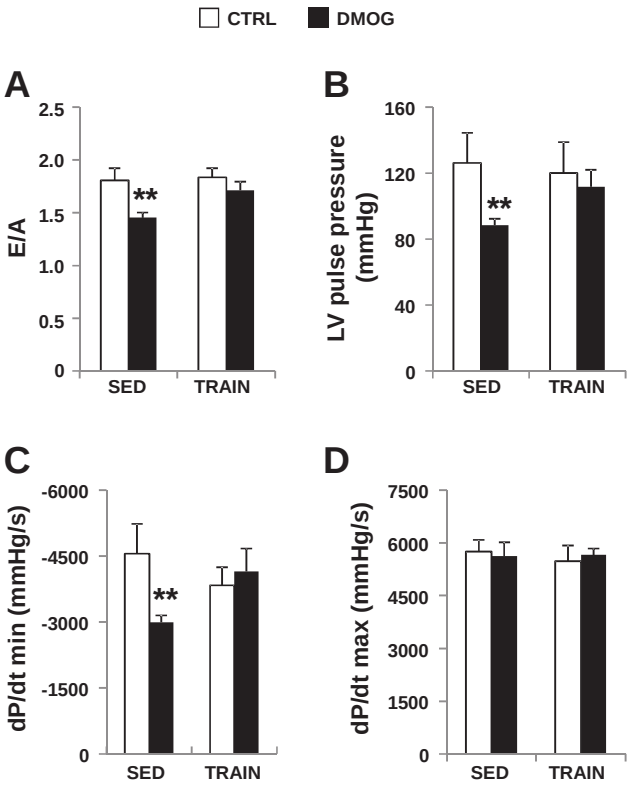

Fig. 4. Effects of DMOG treatment on cardiac function. *A*: E/A ratio in basal condition. *B*: LV pulse pressure. *C*: minimum derivative of LV pressure (dP/d*t* min). *D*: maximum derivative of LV pressure under β-adrenergic stimulation (isoproterenol, 1 mg·kg^{-1}·min^{-1} ip) in SED or trained rats subjected to 1-wk DMOG or NaCl 0.9% (CTRL) treatment. **P ≤ 0.01 vs. corresponding CTRL.

However, we showed that chronic exercise prevented the induction of DMOG-mediated side effects on cardiac muscle. Indeed, the increase in heart weight was observed in sedentary but not in trained rats after DMOG administration. Moreover, the E/A ratio, the LV pulse pressure, and dP/d*t* min were not altered by treatment in trained rats. These results demonstrate that endurance training maintains myocardial function despite DMOG administration. The beneficial role of aerobic exercise on cardiac function is recognized, especially in patients with heart failure (45). Endurance training notably induces the expression of heat shock protein and strengthens defenses against oxidative stress (reviewed in Ref. 2). But, it has also been shown that endurance training improved SERCA-2a-mediated calcium uptake in mice, and this was associated with a significant increase in SERCA-2a protein content (19). This mechanism could, therefore, explain the absence of DMOG-related decrease in LV relaxation with endurance training. Collectively, our findings point to the importance of regular exercise to prevent the induction of deleterious effects consecutive to DMOG treatment.

In line with our results on cardiac muscle, we found similarities between DMOG treatment and hypoxia exposure on skeletal muscle. For example, hypoxia negatively regulates mitochondrial mass and function in skeletal muscle (10, 11, 14, 26). In agreement, we observed a significant reduction in maximal O_2 uptake (relative to mitochondrial mass) and in mitochondrial coupling (RCR) in the gastrocnemius of DMOG-treated rats. Moreover, we did not detect variation of PGC-1 or Tfam gene expression with DMOG administration, suggesting that mitochondrial biogenesis was not altered. Therefore, DMOG effect is rather related to a modification of mitochondrial efficiency rather than mitochondrial content. In contrast, Saxena et al. (38) reported an increase in the expression of mitochondrial biogenesis markers and in mitochondrial density after treatment with $CoCl_2$. Dose and treatment duration are probably of key importance since cobalt has also been shown to cause respiration deficiency in yeast (22). This points to the fact that different types of PHI, such as 2-oxoglutarate antagonist (DMOG) or iron chelator ($CoCl_2$), could have distinct effects on muscle oxidative metabolism. The finding of negative effect of DMOG on mitochondrial respiration observed in isolated mitochondria (present study) or in primary cardiomyocytes (42) has recently been related to HIF-1-independent metabolic disturbance (49). Indeed, once deesterified, DMOG quickly reduced O_2 consumption of rat liver mitochondria likely because of competitive inhibition of mitochondrial

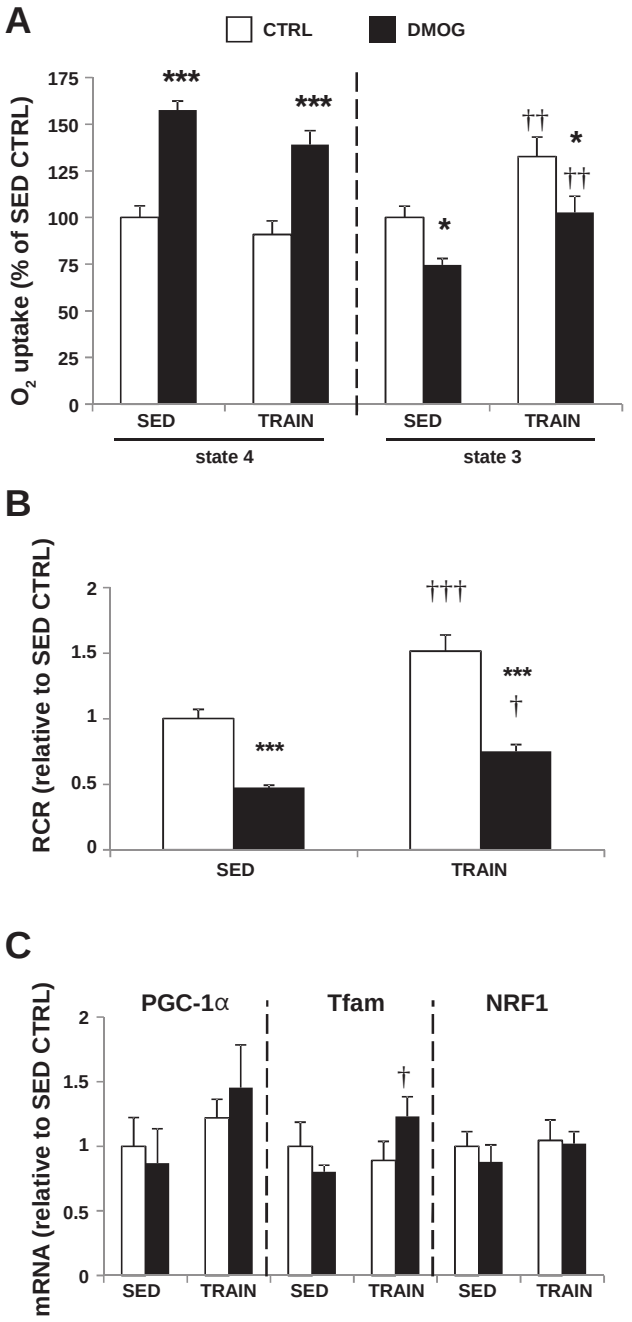

Fig. 5. Effect of DMOG treatment on mitochondrial respiration. *A*: oxygen uptake of state 4 and 3. *B*: respiratory control ratio (RCR // state 3/state 4). *C*: gene expression of transcription factors involved in mitochondrial biogenesis in SED or trained rats subjected to 1 wk of DMOG or NaCl 0.9% (CTRL) treatment. NRF1, nuclear respiratory factor 1; PGC-1 ␣, peroxisome proliferator-activated receptor gamma coactivator 1-␣; Tfam, transcription factor A mitochondrial. **P* ↕ 0.05 and ****P* ↕ 0.001 vs. corresponding CTRL. †*P* ↕ 0.05, ††*P* ↕ 0.01, and †††*P* ↕ 0.001 vs. corresponding SED.

enzymes (such as mitochondrial ␣-ketoglutarate dehydrogenase or isocitrate dehydrogenase). Hypoxia and DMOG can, thus, impair mitochondrial metabolism, but through distinct pathways. Glycolytic metabolism was not markedly altered by DMOG since glycogen stores, HKII, and PFK gene expression remained unaltered. The absence of induction of these known HIF-1 target genes may be surprising, however, the time delay between the last DMOG injection and the measures (96 h) could explain the absence of significant modifications. Although it has been proposed that hypoxia would enhance anaerobic metabolism, a recent review highlighted that skeletal muscle presents only subtle modifications of this metabolism after chronic hypoxia (13).

Besides, endurance training partly reversed side effects of DMOG treatment. Muscle-specific HIF-1 ␣ deletion clearly demonstrates that HIF-1 ␣and endurance training have oppo-

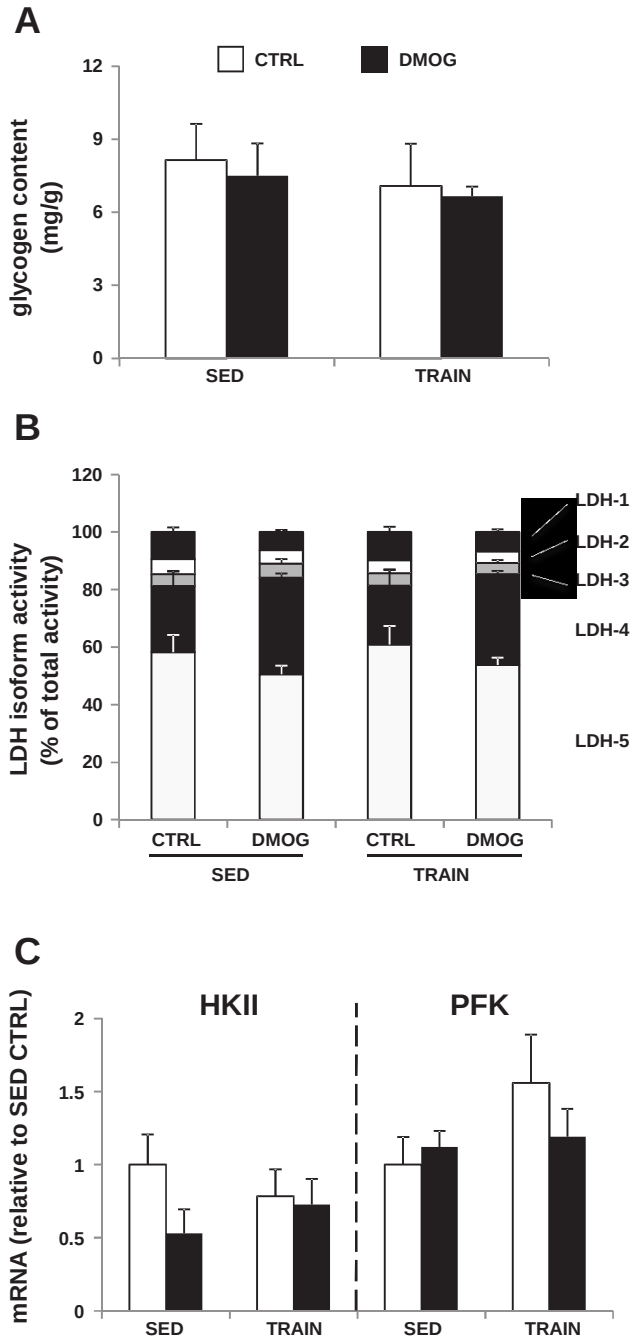

Fig. 6. Effect of DMOG treatment on anaerobic metabolism in plantaris muscle. *A*: glycogen content. *B*: quantification of LDH isoenzymes activity. *C*: gene expression of hexokinase (HK)II and phosphofructokinase (PFK) in SED or trained rats subjected to 1-wk DMOG or NaCl 0.9% (CTRL) treatment.

site effects on skeletal muscle metabolism (27). Moreover, endurance-trained muscle displays enhanced expression of HIF-1 negative regulators (23). Therefore, it is not surprising that endurance training antagonized the HIF-1-dependent effects of DMOG on mitochondrial respiration. However, DMOG treatment led to a similar decrease in state 3 between sedentary and trained rats, and RCR of DMOG-treated trained rats remained slightly but significantly lower than in SED CTRL animals. This probably resulted from HIF-1-independent metabolic competition due DMOG deesterification. Nonetheless, it should be kept in mind that chronic exercise restored maximal O_2 uptake of DMOG-treated muscles to the level of sedentary CTRL rats. As oxidative metabolism capacity is tightly coupled with resistance to fatigue, this could have functional consequences on muscle and subject's fatigability.

Previous studies reported that $CoCl_2$ increased endurance performance during the swimming test in sedentary and trained rats under normoxia (38, 39). Similarly, iron chelation-mediated PHD inhibition by EDHB administration resulted in endurance improvement during running test under severe hypoxic environment (equivalent to 7,400 m) in sedentary mice (18). Accordingly, we observed that DMOG has a positive effect on aerobic performance in sedentary and trained rats under both normoxia and moderate hypoxia (equivalent to 3,000 m). While others showed that this ergogenic effect occurs during endurance exercises (time to exhaustion at a set speed), we showed that DMOG also improved performance during intense aerobic exercise (incremental test for MAV determination). Cardiac output and O_2 delivery are major limiting factors during such efforts (47). However, it had never been investigated whether PHI-induced increase in performance is due to central (O_2 delivery) or peripheral (skeletal muscle) effects. We reported here that DMOG treatment led to a 12.7% increase in MAV that was associated with a similar increase (11.3%) in hemoglobin content. We observed that EDHB was less potent than DMOG to promote erythrocytosis. Our results are in total agreement with the data of Kasiganesan et al. (18), which reported a slight effect on plasma EPO concentration with no change in hematocrit after EDHB treatment. While both EDHB and DMOG stabilize HIF-1 β, DMOG is more efficient for suppressing HIF-1 α hydroxylation on asparagine 803 (43). Since this residue is crucial for HIF-1 transactivation, DMOG appears as a powerful activator of HIF-1 by enhancing stabilization and transcriptional activity of HIF-1β. Despite this, ex vivo muscle contractile properties, and particularly resistance to fatigue, were unaltered by DMOG administration. Collectively, these results strongly suggest that PHI-mediated increase in aerobic performance is mainly due to central, via an increase in O_2 delivery, rather than a peripheral effect.

Conclusions and Perspectives

Hypoxic preconditioning is of clinical interest for the prevention of ischemic complications. We confirm that PHI treatment activates erythrocytosis, although all molecules are not equally potent for such activation. Moreover, DMOG has recently been used to promote bone regeneration (48) and odontoblast maturation (33) or to prevent oxygen-induced retinopathy (44). Therefore, DMOG would be clinically relevant for a broad range of patients, and some of PHIs have already been used in humans (32). Endurance athletes may also be tempted to use DMOG to improve their performances, as recently revealed. However, the potential side effects of such therapy remain poorly considered. For example, $CoCl_2$ was used to improve exercise performance (38, 39) and limit myocardial infarction (5), whereas it could have toxic effects in animals (9). Here, we provide evidence that the 2-oxoglutarate antagonist DMOG has a negative effect on myocardial function in healthy rats. Efforts are made to develop more selective PHI compared with DMOG that may also inhibit other 2-oxoglutarate-dependentdioxygenases, including the collagen-modifying hydroxylases. However, alteration of heart function with DMOG appears to be dependent upon HIF-1α activation, and specific PHI would probably lead to the same effects. One may also suppose that the deleterious influence of PHI would be potentiated in patients with already poor physical condition. Fortunately, these detrimental effects can be reversed by regular aerobic exercise. In conclusion, our work underlines the need to take into consideration the potential side effects of PHI when administrated to patients and the interest of physical activity to prevent these effects.

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Present address of G. Begue: Human Performance Laboratory, Ball State University, 2000 W. University Ave., Muncie, IN 47306.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: F.B.F., C.R., G.M., and G.P. conception and design of research; F.B.F., F.A.B., B.P., G.B., B.C., C.R., G.M., and G.P. performed experiments; F.B.F., F.A.B., B.P., G.B., B.C., C.R., G.M., and G.P. analyzed data; F.B.F., F.A.B., B.P., G.B., B.C., C.R., G.M., and G.P. interpreted results of experiments; F.B.F. prepared figures; F.B.F. drafted manuscript; F.B.F., C.R., G.M., and G.P. edited and revised manuscript; F.B.F., F.A.B., B.P., G.B., B.C., C.R., G.M., and G.P. approved final version of manuscript.

REFERENCES

1. **Andre L, Boissière J, Reboul C, Perrier R, Zalvidea S, Meyer G, Thireau J, Tanguy S, Bideaux P, Hayot M, Boucher F, Obert P, Cazorla O, Richard S.** Carbon monoxide pollution promotes cardiac remodeling and ventricular arrhythmia in healthy rats. *Am J Respir Crit Care Med* 181: 587–595, 2010.
2. **Ascensão A, Ferreira R, Magalhães J.** Exercise-induced cardioprotection— biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. *Int J Cardiol* 117: 16 –30, 2007.
3. **Asikainen TM, Ahmad A, Schneider BK, Ho WB, Arend M, Brenner M, Günzler V, White CW.** Stimulation of HIF-1 β, HIF-2 β and VEGF by prolyl 4-hydroxylase inhibition in human lung endothelial and epithelial cells. *Free Radic Biol Med* 38: 1002–1013, 2005.
4. **Bekeredjian R, Walton CB, MacCannell KA, Ecker J, Kruse F, Outten JT, Sutcliffe D, Gerard RD, Bruick RK, Shohet RV.** Conditional HIF-1α expression produces a reversible cardiomyopathy. *PloS One* 5: e11693, 2010.
5. **Belaidi E, Beguin PC, Levy P, Ribuot C, Godin-Ribuot D.** Delayed myocardial preconditioning induced by cobalt chloride in the rat: HIF-1 α and iNOS involvement. *Fundam Clin Pharmacol* 26: 454 –462, 2012.
6. **Belanger AJ, Luo Z, Vincent KA, Akita GY, Cheng SH, Gregory RJ, Jiang C.** Hypoxia-inducible factor 1 mediates hypoxia-induced cardiomyocyte lipid accumulation by reducing the DNA binding activity of peroxisome proliferator-activated receptor α/retinoid X receptor. *Biochem Biophys Res Commun* 364: 567–572, 2007.

7. **Britto FA, Begue G, Rossano B, Docquier A, Vernus B, Sar C, Ferry A, Bonnieu A, Ollendorff V, Favier FB.** REDD1 deletion prevents dexamethasone-induced skeletal muscle atrophy. *Am J Physiol Endocrinol Metab* 307: E983–E993, 2014.
8. **Chen RL, Ogunshola OO, Yeoh KK, Jani A, Papadakis M, Nagel S, Schofield CJ, Buchan AM.** HIF prolyl hydroxylase inhibition prior to transient focal cerebral ischaemia is neuroprotective in mice. *J Neurochem* 131: 177–189, 2014.
9. **Domingo JL.** Cobalt in the environment and its toxicological implications. *Rev Environ Contam Toxicol* 108: 105–132, 1989.
10. **Galbès O, Goret L, Caillaud C, Mercier J, Obert P, Candau R, Py G.** Combined effects of hypoxia and endurance training on lipid metabolism in rat skeletal muscle. *Acta Physiol (Oxf)* 193: 163–173, 2008.
11. **Gamboa JL, Andrade FH.** Muscle endurance and mitochondrial function after chronic normobaric hypoxia: contrast of respiratory and limb muscles. *Pflügers Arch* 463: 327–338, 2012.
12. **Greijer AE, van der Groep P, Kemming D, Shvarts A, Semenza GL, Meijer GA, van de Wiel MA, Belien a J. M, van Diest PJ, van der Wall E.** Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). *J Pathol* 206: 291–304, 2005.
13. **Horscroft JA, Murray AJ.** Skeletal muscle energy metabolism in environmental hypoxia: climbing towards consensus. *Extreme Physiol Med* 3, 19, 2014.
14. **Howald H, Hoppeler H.** Performing at extreme altitude: muscle cellular and subcellular adaptations. *Eur J Appl Physiol* 90: 360 –364, 2003.
15. **Hsieh MM, Linde NS, Wynter A, Metzger M, Wong C, Langsetmo I, Lin A, Smith R, Rodgers GP, Donahue RE, Klaus SJ, Tisdale JF.** HIF prolyl hydroxylase inhibition results in endogenous erythropoietin induction, erythrocytosis, and modest fetal hemoglobin expression in rhesus macaques. *Blood* 110: 2140 –2147, 2007.
16. **Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG.** HIF␣ targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* 292: 464 –468, 2001.
17. **Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ.** Targeting of HIF-␣ to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292: 468 –472, 2001.
18. **Kasiganesan H, Sridharan V, Wright G.** Prolyl hydroxylase inhibitor treatment confers whole-animal hypoxia tolerance. *Acta Physiol (Oxf)* 190: 163–169, 2007.
19. **Kemi OJ, Ceci M, Condorelli G, Smith GL, Wisloff U.** Myocardial sarcoplasmic reticulum Ca^{2+} ATPase function is increased by aerobic interval training. *Eur J Cardiovasc Prev Rehabil* 15: 145–148, 2008.
20. **Lanza IR, Nair KS.** Functional assessment of isolated mitochondria in vitro. *Methods Enzymol* 457: 349 –372, 2009.
21. **Lei L, Mason S, Liu D, Huang Y, Marks C, Hickey R, Jovin IS, Pypaert M, Johnson RS, Giordano FJ.** Hypoxia-inducible factor-dependent degeneration, failure, and malignant transformation of the heart in the absence of the von Hippel-Lindau protein. *Mol Cell Biol* 28: 3790 –3803, 2008.
22. **Lindegren CC, Nagai S, Nagai H.** Induction of respiratory deficiency in yeast by manganese, copper, cobalt and nickel. *Nature* 182: 446 –448, 1958.
23. **Lindholm ME, Fischer H, Poellinger L, Johnson RS, Gustafsson T, Sundberg CJ, Rundqvist H.** Negative regulation of HIF in skeletal muscle of elite endurance athletes: a tentative mechanism promoting oxidative metabolism. *Am J Physiol Regul Integr Comp Physiol* 307: R248–R255, 2014.
24. **Long X, Boluyt MO, Hipolito ML, Lundberg MS, Zheng JS, O'Neill L, Cirielli C, Lakatta EG, Crow MT.** p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. *J Clin Invest* 99: 2635–2643, 1997.
25. **Lo S, Russell JC, Taylor AW.** Determination of glycogen in small tissue samples. *J Appl Physiol* 28: 234 –236, 1970.
26. **Magalhães J, Ascensão A, Soares JMC, Ferreira R, Neuparth MJ, Marques F, Duarte JA.** Acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in mouse skeletal muscle. *J Appl Physiol (1985)* 99: 1247–1253, 2005.
27. **Mason SD, Rundqvist H, Papandreou I, Duh R, McNulty WJ, Howlett RA, Olfert IM, Sundberg CJ, Denko NC, Poellinger L, Johnson RS.** HIF-1␣ in endurance training: suppression of oxidative metabolism. *Am J Physiol Regul Integr Comp Physiol* 293: R2059 –R2069, 2007.
28. **McCullagh KJ, Poole RC, Halestrap AP, Tipton KF, O'Brien M, Bonen A.** Chronic electrical stimulation increases MCT1 and lactate uptake in red and white skeletal muscle. *Am J Physiol Endocrinol Metab* 273: E239 –E246, 1997.
29. **Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin WG.** Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. *Blood* 111: 3236 –3244, 2008.
30. **Moslehi J, Minamishima YA, Shi J, Neuberg D, Charytan DM, Padera RF, Signoretti S, Liao R, Kaelin WG.** Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. *Circulation* 122: 1004 –1016, 2010.
31. **Ockaili R, Natarajan R, Salloum F, Fisher BJ, Jones D, Fowler AA, Kukreja RC.** HIF-1 activation attenuates postischemic myocardial injury: role for heme oxygenase-1 in modulating microvascular chemokine generation. *Am J Physiol Heart Circ Physiol* 289: H542–H548, 2005.
32. **Olson E, Demopoulos L, Haws TF, Hu E, Fang Z, Mahar KM, Qin P, Lepore J, Bauer TA, Hiatt WR.** Short-term treatment with a novel HIF-prolyl hydroxylase inhibitor (GSK1278863) failed to improve measures of performance in subjects with claudication-limited peripheral artery disease. *Vasc Med* 19: 473–482, 2014.
33. **Rahman SU, Lee MS, Baek JH, Ryoo HM, Woo KM.** The prolyl hydroxylase inhibitor dimethyloxalylglycine enhances dentin sialophoshoprotein expression through VEGF-induced Runx2 stabilization. *PloS One* 9: e112078, 2014.
34. **Reboul C, Tanguy S, Dauzat M, Obert P.** Altitude negates the benefits of aerobic training on the vascular adaptations in rats. *Med Sci Sports Exerc* 37: 979 –985, 2005.
35. **Reboul C, Tanguy S, Juan JM, Dauzat M, Obert P.** Cardiac remodeling and functional adaptations consecutive to altitude training in rats: implications for sea level aerobic performance. *J Appl Physiol (1985)* 98: 83–92, 2005.
36. **Ripamonti M, Viganò A, Moriggi M, Milano G, von Segesser LK, Samaja M, Gelfi C.** Cytochrome-*c* oxidase expression in chronic and intermittent hypoxia rat gastrocnemius muscle quantitated by CE. *Electrophoresis* 27: 3897–3903, 2006.
37. **Sang N, Fang J, Srinivas V, Leshchinsky I, Caro J.** Carboxyl-terminal transactivation activity of hypoxia-inducible factor 1 alpha is governed by a von Hippel-Lindau protein-independent, hydroxylation-regulated association with p300/CBP. *Mol Cell Biol* 22: 2984 –2992, 2002.
38. **Saxena S, Shukla D, Bansal A.** Augmentation of aerobic respiration and mitochondrial biogenesis in skeletal muscle by hypoxia preconditioning with cobalt chloride. *Toxicol Appl Pharmacol* 264: 324 –334, 2012.
39. **Saxena S, Shukla D, Saxena S, Khan YA, Singh M, Bansal A, Sairam M, Jain SK.** Hypoxia preconditioning by cobalt chloride enhances endurance performance and protects skeletal muscles from exercise-induced oxidative damage in rats. *Acta Physiol* 200: 249 –263, 2010.
40. **Semenza GL.** HIF-1, O_2, and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 107: 1–3, 2001.
41. **Slot IGM, Schols AMWJ, Vosse BAH, Kelders MCJM, Gosker HR.** Hypoxia differentially regulates muscle oxidative fiber type and metabolism in a HIF-1 ␣dependent manner. *Cell Signal* 26: 1837–1845, 2014.
42. **Sridharan V, Guichard J, Bailey RM, Kasiganesan H, Beeson C, Wright GL.** The prolyl hydroxylase oxygen-sensing pathway is cytoprotective and allows maintenance of mitochondrial membrane potential during metabolic inhibition. *Am J Physiol Cell Physiol* 292: C719 –C728, 2007.
43. **Tian YM, Yeoh KK, Lee MK, Eriksson T, Kessler BM, Kramer HB, Edelmann MJ, Willam C, Pugh CW, Schofield CJ, Ratcliffe PJ.** Differential sensitivity of hypoxia inducible factor hydroxylation sites to hypoxia and hydroxylase inhibitors. *J Biol Chem* 286: 13041–13051, 2011.
44. **Trichonas G, Lee TJ, Hoppe G, Au J, Sears JE.** Prolyl hydroxylase inhibition during hyperoxia prevents oxygen-induced retinopathy in the rat 50/10 model. *Invest Ophthalmol Vis Sci* 54: 4919 –4926, 2013.
45. **Ventura-Clapier R, Mettauer B, Bigard X.** Beneficial effects of endurance training on cardiac and skeletal muscle energy metabolism in heart failure. *Cardiovasc Res* 73: 10 –18, 2007.
46. **Vogler M, Zieseniss A, Hesse AR, Levent E, Tiburcy M, Heinze E, Burzlaff N, Schley G, Eckardt KU, Willam C, Katschinski DM.** Pre- and post-conditional inhibition of prolyl-4-hydroxylase domain enzymes protects the heart from an ischemic insult. *Pflügers Arch* 467: 2141–2149, 2015.
47. **Wagner PD.** New ideas on limitations to VO2max. *Exerc Sport Sci Rev* 28: 10 –14, 2000.
48. **Woo KM, Jung HM, Oh JH, Rahman SU, Kim SM, Baek JH, Ryoo HM.** Synergistic effects of dimethyloxalylglycine and butyrate incorporated into ␣calcium sulfate on bone regeneration. *Biomaterials* 39: 1–14, 2015.
49. **Zhdanov AV, Okkelman IA, Collins FWJ, Melgar S, Papkovsky DB.** A novel effect of DMOG on cell metabolism: direct inhibition of mitochondrial function precedes HIF target gene expression. *Biochim Biophys Acta* 1847: 1254 –1266, 2015.