

Acute in vitro hypoxia and high-altitude (4,559 m) exposure decreases leukocyte oxygen consumption

Vitalie Faoro, Bruno Fink, Sarah Taudorf, Christoph Dehnert, Marc M. Berger, Erik R. Swenson, Damian M. Bailey, Peter Bärtsch and Heimo Mairbäurl

Am J Physiol Regul Integr Comp Physiol 300:R32-R39, 2011. First published 20 October 2010; doi: 10.1152/ajpregu.00413.2010

You might find this additional info useful...

This article cites 40 articles, 21 of which you can access for free at:
<http://ajpregu.physiology.org/content/300/1/R32.full#ref-list-1>

Updated information and services including high resolution figures, can be found at:
<http://ajpregu.physiology.org/content/300/1/R32.full>

Additional material and information about *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* can be found at:
<http://www.the-aps.org/publications/ajpregu>

This information is current as of December 13, 2012.

American Journal of Physiology - Regulatory, Integrative and Comparative Physiology publishes original investigations that illuminate normal or abnormal regulation and integration of physiological mechanisms at all levels of biological organization, ranging from molecules to humans, including clinical investigations. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2011 the American Physiological Society. ISSN: 0363-6119, ESSN: 1522-1490. Visit our website at <http://www.the-aps.org/>.

CALL FOR PAPERS | *Mitochondrial Function/Dysfunction in Health and Disease*

Acute in vitro hypoxia and high-altitude (4,559 m) exposure decreases leukocyte oxygen consumption

Vitalie Faoro,^{1,2} Bruno Fink,³ Sarah Taudorf,⁴ Christoph Dehnert,¹ Marc M. Berger,⁵ Erik R. Swenson,⁶ Damian M. Bailey,⁷ Peter Bärtsch,¹ and Heimo Mairbäurl¹

¹Medical Clinic VII, Sports Medicine, University Hospital Heidelberg, Heidelberg, Germany; ²Department of Pathophysiology, University of Brussels, Brussels, Belgium; ³Noxygen Science Transfer & Diagnostics, Germany; ⁴Centre of Inflammation and Metabolism, Rigshospitalet, Copenhagen, Denmark; ⁵Department of Anesthesiology, University of Heidelberg, Heidelberg, Germany; ⁶Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, Washington; and ⁷Neurovascular Research Laboratory, Faculty of Health, Science and Sport, University of Glamorgan, Glamorgan, UK

Submitted 23 June 2010; accepted in final form 11 October 2010

Faoro V, Fink B, Taudorf S, Dehnert C, Berger MM, Swenson ER, Bailey DM, Bärtsch P, Mairbäurl H. Acute in vitro hypoxia and high-altitude (4559 m) exposure decreases leukocyte oxygen consumption. *Am J Physiol Regul Integr Comp Physiol* 300: R32–R39, 2011. First published October 20, 2010; doi:10.1152/ajpregu.00413.2010.—Hypoxia impairs metabolic functions by decreasing activity and expression of ATP-consuming processes. To separate hypoxia from systemic effects, we tested whether hypoxia at high altitude affects basal and PMA-stimulated leukocyte metabolism and how this compares to acute (15 min) and 24 h of in vitro hypoxia. Leukocytes were prepared at low altitude and ~24 h after arrival at 4559 m. Mitochondrial oxygen consumption (JO₂) was measured by respirometry, oxygen radicals by electron spin resonance spectroscopy, both at a P_O₂ = 100 mmHg (JO_{2,100}) and 20 mmHg (JO_{2,20}). Acute hypoxia of leukocytes decreased JO₂ at low altitude. Exposure to high altitude decreased JO_{2,100}, whereas JO_{2,20} was not affected. Acute hypoxia of low-altitude samples decreased the activity of complexes I, II, and III. At high altitude, activity of complexes I and III were decreased when measured in normoxia. Stimulation of leukocytes with PMA increased JO_{2,100} at low (twofold) and high altitude (five-fold). At both locations, PMA-stimulated JO₂ was decreased by acute hypoxia. Basal and PMA-stimulated reactive oxygen species (ROS) production were unchanged at high altitude. Separate in vitro experiments performed at low altitude show that ~75% of PMA-induced increase in JO₂ was due to increased extra-mitochondrial JO₂ (JO_{2,res}; in the presence of rotenone and antimycin A). JO_{2,res} was doubled by PMA. Acute hypoxia decreased basal JO_{2,res} by ~70% and PMA-stimulated JO_{2,res} by about 50% in cells cultured in normoxia and hypoxia (1.5% O₂; 24 h). Conversely, 24 h in vitro hypoxia decreased mitochondrial JO_{2,100} and JO_{2,20}, extra-mitochondrial, basal, and PMA-stimulated JO₂ were not affected. These results show that 24 h of high altitude but not 24 h in vitro hypoxia decreased basal leukocyte metabolism, whereas PMA-induced JO₂ and ROS formation were not affected, indicating that prolonged high-altitude hypoxia impairs mitochondrial metabolism but does not impair respiratory burst. In contrast, acute hypoxia impairs respiratory burst at either altitude.

energy metabolism; respiratory burst; NADPH oxidase; hypoxia

A DECREASED OXYGEN SUPPLY, such as in cardiovascular disorders (e.g., congestive heart failure, ischemia), decreased oxygen transport capacity, pulmonary diseases (e.g., chronic obstructive pulmonary disease), and decreased inspired P_O₂, such as at high altitude, impairs cellular functions by decreasing cellular activity, metabolism, protein synthesis, and ion transport (21, 22, 36, 39, 40). Mitochondrial respiration is decreased even at values of P_O₂ between 10 and 60 mmHg, which do not limit mitochondrial respiration, a process termed “oxygen conformance” (21, 36). These energy-sparing mechanisms are initiated within minutes of exposure to hypoxia. Below an extracellular P_O₂ of about 10 mmHg (21), cells become ATP depleted by a decrease in mitochondrial oxidative metabolism. These changes can cause dysfunction of mitochondria and ischemic or hypoxic cell injury with subsequent structural alterations (9). The mechanisms causing these rapid changes are not fully understood.

After short-term exposure to hypoxia (15 min), decreased oxygen consumption and mitochondrial activity can fully be restored by a brief period of reoxygenation (21). In contrast, we found that changes induced by 24 h of hypoxia are not readily reversible. This process might indicate decreased expression of genes encoding for enzymes involved in pathways with a high energy demand, as well as a decreased expression of mitochondrial enzymes. On the other hand, there is a stimulation of genes by the hypoxia-induced transcription factor (HIF) aimed at improving anaerobic metabolism and substrate supply, as well as restoring tissue oxygen supply (38).

Hypoxia also decreases leukocyte oxygen consumption (14). In contrast, leukocyte oxygen demand is increased when cells are stimulated by initiation of respiratory burst (14), which increases leukocyte ROS production by NADPH oxidase (43). Since this process requires oxygen, it might be impaired in hypoxia due to substrate limitation (14). A decrease in nitroblue tetrazolium reduction (24), decreased diacylglycerol formation, modulation of the PIP₂-PLC pathway, and altered stimulation by interleukins (8) indicate impaired leukocyte function in hypoxia. Furthermore the respiratory burst of neutrophils has also been shown to decrease in hypoxia (14), which was paralleled by a decreased activity of O₂^{•-} forming enzymes (14). Subsequently, wound healing and bacterial killing might be impaired (2). Hypoxia delays leukocyte apoptosis,

Address for reprint requests and other correspondence: H. Mairbäurl, Medical Clinic VII, Sports Medicine, Univ. Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany (e-mail: heimo.mairbaeurl@med.uni-heidelberg.de).

a major mechanism resolving inflammation (19), which might be due to a hypoxia-induced increase in macrophage inflammatory protein-1 β (41). It is unclear, however, whether impaired respiratory burst and delayed apoptosis are related processes. A decrease in ROS production in hypoxia has been found in other cell types as well (11, 30). However, results are controversial since hypoxia has also been shown to induce the formation of oxygen radicals (ROS) by the mitochondrial electron transfer chain, at least when O₂ becomes very low (4).

Most results on impaired leukocyte function were obtained during short-term, *in vitro* exposure to hypoxia. If these *in vitro* results apply to the *in vivo* situation, leukocyte function can be expected to be impaired in diseases and situations that are classically characterized by arterial hypoxemia. Using hypoxia of high altitude (4,559 m) as a model, we tested, therefore, whether *in vivo* hypoxia impairs leukocyte metabolism, mitochondrial function, and ROS production. To separate direct effects of hypoxia from systemic effects caused by high-altitude hypoxia, we compared these results with 24-h *in vitro* hypoxia of leukocytes collected from normoxic individuals. In both studies, cellular respiration and ROS production were also measured upon stimulation with PMA to test whether hypoxia affected the capacity of respiratory burst. All measurements were performed on cell suspensions equilibrated to a normoxic and hypoxic gas, which allows distinguishing effects of acute oxygen deprivation from adjustments caused by prolonged hypoxia.

MATERIALS AND METHODS

Chemicals/reagents. Ficoll-Paque plus was obtained from Amersham Biosciences (Uppsala, Sweden). HEPES was obtained from Gibco (Gibco, Karlsruhe, Germany). Rotenone, antimycin A, PMA, malate, glutamate, succinate, ADP, sucrose, and basic reagents were obtained from Sigma Chemicals (Sigma-Aldrich, Munich, Germany), and digitonin and fatty acid free BSA were obtained from Serva Chemicals (Serva, Heidelberg, Germany). Decylubiquinone was a gift from Dr. Okun (Children's Hospital, University of Heidelberg, Heidelberg, Germany).

Study population. Mountaineers (2 females, 13 males; 37 \pm 11 years) without and with previously documented high-altitude pulmonary edema (HAPE) and acute mountain sickness (AMS) were recruited through announcements in the journals of the Swiss and German Alpine Clubs and participated in this high-altitude study after written informed consent in a study on the pathophysiology of increased lung water at high altitude. They represent a subset of the individuals participating in a more extensive study on respiratory function at high altitude published recently (7). The study protocol was approved by the ethics committee of the University of Heidelberg. AMS was assessed using the Lake Louise Scoring System (32), and the cerebral symptoms of AMS (AMS-C) score of the Environmental Symptoms Questionnaire (33). AMS was diagnosed if subjects had a Lake Louise score of >4 and an AMS-C score of \geq 0.70 in the morning of the second day at 4,559 m. HAPE was assessed by chest radiography (TRS, Siemens, Stockholm, Sweden) and clinical evaluation. None of the participants was considered preacclimatized since they did not spend more than 4 nights above 2,500 m within 30 days before the study at high altitude.

Baseline evaluations were performed in Heidelberg (110 m) 2 to 4 wk prior to high-altitude exposure. Subjects, in groups of three every 3 days, were transported by cable car to 3,200 m and then trekked to 3,611 m where they spent the night (Capanna Gnifetti). In the morning, they ascended to 4,559 m (Capanna Regina Margherita) with a certified mountain guide. At high altitude (HA), blood samples

from one subject per arriving group was obtained for leukocyte preparation at ~0700 following the first overnight stay, which was ~20 h after arrival at 4,559 m.

Preparation of leukocytes. Blood (18 ml, anticoagulated with K-Edta, Sarstaedt, Germany) was collected via a central venous catheter inserted from the antecubital vein for leukocyte preparation and RNA isolation. Blood was centrifuged at 1,000 g at 4°C to remove plasma. The buffy coat was collected with Pasteur pipettes, suspended in 10 ml PBS and layered on top of 20 ml Ficoll-Paque plus (Amersham Biosciences) using 50-ml Falcon tubes. After centrifugation at 400 g for 30 min at 15°C, the supernatant was removed, and polymorphonuclear leukocytes were collected at the gradient interface, suspended in 40 ml PBS, and centrifuged at 400 g for 10 min at 4°C. Packed cells were suspended in a medium composed (in mM) of 135 NaCl, 5 KCl, 10 glucose, 1 Na₂HPO₄, 1 MgCl₂, 1 CaCl₂, 10 HEPES, 1 mg/ml BSA, pH 7.4 at 37°C. This cell suspension was stored at 10°C until use for measurements of oxygen consumption and ROS production using respirometry and electron spin resonance (ESR) spectroscopy, respectively.

Respirometry. Mitochondrial oxygen consumption (JO₂) was measured in a respirometer using Clark-type electrodes (Oxygraph, OROBOROS Instruments, Innsbruck, Austria) at 37°C, as described previously (21). JO₂ was calculated from the time derivative of the decrease in oxygen content in the closed chamber, which was corrected for the response time of the oxygen sensor and for instrumental background (back diffusion of oxygen, oxygen consumption by the oxygen sensor). JO₂ was corrected for citrate synthase, an enzyme located in the mitochondrial matrix, which is commonly used as a marker for the cellular content of intact mitochondria (21, 23).

Measurements of JO₂ and ROS formation were performed at the same PO₂ at low and at high altitude. "Normoxia" was defined as a PO₂ of about 100 mmHg representing an average value of PO₂ in arterial blood at low altitude. At low altitude, this was achieved by equilibrating the samples in the respirometer with a gas composed of 14% O₂ and 86% N₂ before closing the chamber. To achieve the same PO₂ at high altitude, the gas contained 25% O₂ and 75% N₂. "Acute hypoxia" (PO₂ = 20 mmHg) during the measurement in the respirometer was introduced by decreasing the PO₂ in the respirometer by equilibration with a gas composed of 2.8% O₂ (97.2% N₂) and 5% O₂ and 95% N₂ at low and high altitude, respectively. At this PO₂, mitochondrial respiration is not oxygen limited (21).

The same buffer as used for preparing cell suspensions was warmed to 37°C in the respirometer chamber and equilibrated with room air for calibration of the electrode. Cell suspension was added, and the PO₂ was adjusted to 100 mmHg (normoxia) with the respective gas (see also Ref. 21). Then, the system was closed to start the oxygen consumption measurement (JO_{2,100}). After a stable reading was achieved, the chamber was opened, and the cell suspension was equilibrated with the respective hypoxic gas mix. Equilibration took about 5 min. The system was closed again to determine oxygen consumption in acute hypoxia (JO_{2,20}). Measurements were repeated in cell suspensions to which PMA (200 nM final concentration) was added during equilibration with the normoxic gas in the respirometer. PMA is known to stimulate NADPH oxidase (43).

Permeabilization of cells. In the altitude study, the activity of complexes I, II, and III of the mitochondrial electron transfer chain (mETC) was measured after permeabilization of the plasma membrane with the nonionic detergent digitonin to allow entry of complex-specific substrates (16, 20). Permeabilization was performed in the cuvette of the respirometer by adding 5 μ g digitonin to 2.5 ml of cell suspension. This dose was evaluated in separate titration experiments by visualizing trypan blue staining and succinate-dependent respiration (not shown). Oxygen consumption was then measured in the presence of digitonin. The permeabilization medium contained (in mM) 75 sucrose, 100 KCl, 10 KH₂PO₄, 0.5 EDTA, 5 MgCl₂, 20 Tris-HCl, and 1 mg/ml BSA (fatty acid free). Substrates and inhibitors of mitochondrial respiration were added as required to measure the

activity of the mETC complexes I, II, and III at the following concentrations: 2 mM malate, 5 mM glutamate, 2 mM ADP, 2.5 μ M rotenone, 10 mM succinate, 50 μ M decylubiquinone, and 1 mM antimycin A (21). Measurements were performed in normoxia and hypoxia as described above for intact cells.

Measurement of ROS formation by leukocytes. Because leukocytes contribute to the respiratory burst, it was of importance to know whether acute exposure of the cells to in vitro hypoxia and exposure to high altitude would affect basal and PMA-stimulated ROS formation. The medium (in mM) 135 NaCl, 5 KCl, 10 glucose, 1 Na_2HPO_4 , 1 MgCl_2 , 1 CaCl_2 , 10 HEPES, 1 mg/ml BSA, pH 7.4 at 37°C also contained 25 μ M desferrioxamine and 5 μ M diethyldithiocarbamate to control iron-dependent ROS formation during the incubation. Cells were prepared for oxygen consumption measurements, as described above, transferred to the respirometer, and equilibrated with normoxic gas in the absence and presence of 200 nM PMA. The cell-permeable spin probe CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine; 500 μ M final concentration; Noxygen, Elzach, Germany) was added. Upon reaction with ROS, this spin probe is transformed into a stable CM^\bullet radical that can be detected by X-band ESR spectroscopy (10). An initial sample of the cell suspension (250 μ l) was transferred into a piece of a 1-ml plastic syringe, closed with Parafilm, and frozen immediately in liquid nitrogen. The respirometer was closed, and the cells were incubated for 10 min, during which time, oxygen consumption was also recorded. Then a second sample was taken and frozen immediately. Measurements and sampling were repeated in cells equilibrated to the hypoxic gas.

The CM^\bullet radical concentration was measured by ESR spectroscopy (26). Spectra were recorded at -196°C using a benchtop ESR-spectrometer (MS200, Noxygen Science Transfer & Diagnostics) using the following settings: field sweep, 120 G; microwave frequency, 9.78 GHz; microwave power, 1 mW; modulation amplitude, 3 G; sweep time 120 s; receiver gain, 500. Frozen standard solutions were used for quantification (Noxygen Science Transfer & Diagnostics).

In vitro hypoxia. In a separate series of measurements, which were performed in Heidelberg only, leukocytes from individuals who did not participate in the high-altitude study and who were not exposed to hypoxia were prepared as described above ($n = 6$). Cells were then suspended in RMPI containing 20 mM HEPES, penicillin, and streptomycin, and 10% FCS and were kept in tissue culture in normoxia and in normobaric hypoxia (1.5% O_2 , 5% CO_2 , rest N_2) for 24 h. After incubation, cells were washed and suspended in buffer (see above) for respiration measurements. Extra-mitochondrial oxygen consumption was determined as an indicator of the activity of NADPH oxidase in the absence and presence of PMA after inhibition of complex I and complex III respiration. After equilibration with the normoxic gas, DMSO or PMA was added in the absence and presence of rotenone (0.5 μ M) and antimycin A (2.5 μ M), and oxygen consumption was recorded at a P_{O_2} of 100 mmHg and 20 mmHg, as described above, to distinguish between mitochondrial and residual (extra-mitochondrial) oxygen consumption, the latter being used as a surrogate marker of NADPH oxidase.

Definitions. In this paper, the prefix CO- refers to the study on leukocytes from normoxic controls only, the prefixes LA- and HA- refer to the measurements in the prealtitude and high-altitude test, respectively. The term JO_2 is used to denote oxygen consumption measured with the respirometer. In either study, control and the high-altitude experiment, JO_2 was measured after equilibrating the buffer in the respirometer to a normoxic gas to determine JO_2 at a P_{O_2} of 100 mmHg ($\text{JO}_{2,100}$) and after equilibration to a hypoxic gas ($\text{P}_{\text{O}_2} = 20$ mmHg) to obtain $\text{JO}_{2,20}$, independent of the altitude at which measurements were performed. Mitochondrial oxygen consumption in the control study was the proportion of JO_2 inhibited by rotenone and antimycin A ($\text{CO-JO}_{2,m,100}$ and $\text{CO-JO}_{2,m,20}$). Residual oxygen consumption was the JO_2 measured in the presence of rotenone and antimycin A ($\text{CO-JO}_{2,res,100}$ and $\text{CO-JO}_{2,res,20}$). Subscripts C1, C2,

and C3 refer to the respiration of the complexes I, II, and III, respectively, of the mitochondrial electron transfer chain.

Biochemical analysis. For RT-PCR, cells obtained at low and high altitude were lysed using the RLT reagent (Qiagen, Gaithersburg, MD, USA). Total RNA was isolated from the lysates with the RNeasy mini kit (Qiagen), according to the manufacturer's instructions. RNA (0.1 μ g) was transcribed with Superscript II reverse transcriptase (Invitrogen, Life Technologies, Darmstadt, Germany) using random hexamer primers (Roche, Mannheim, Germany). Real-time quantitative PCR was performed in the LightCycler (Roche). The QuantiTect SYBR Green PCR kit was used with QuantiTect primers for the detection of COX-1 and COX-2 (QuantiTect, Qiagen, Hilden, Germany). To test for the specificity of PCR amplification, PCR products were separated by agarose gel electrophoresis and stained with Gelstar (BMA, ME). PCR products showed single bands of the predicted size (not shown). Standards for quantification of PCR products were prepared by conventional PCR using the primers mentioned above and eluates of PCR products from agarose gels. 28S-rRNA was used to control for differences in the efficacy of reverse transcription.

Statistics. Results are shown as mean values \pm SD or SE of the number of experiments indicated in the figure legends. Statistical analysis was done by Student's *t*-tests, one-way ANOVA and Tukey post hoc tests using SigmaPlot (SYSTAT, Erkrath, Germany) as appropriate. Level of significance was $P \leq 0.05$.

RESULTS

From the individuals, whose leukocytes were studied, nine individuals had AMS; three of those also had HAPE. This relatively high incidence of HAPE is due to the fact that approximately half of the individuals whose leukocytes were studied were susceptible to HAPE. We analyzed whether altered leukocyte metabolism was related to severity of AMS. However, there was no correlation between leukocyte metabolism and the Lake Louise score or AMS-C (not shown).

Intact cells, high-altitude study. Citrate synthase (CS), a commonly used marker of mitochondrial density (23), was used to normalize JO_2 . CS was 2.27 ± 1.05 mU/ml of leukocyte suspension in low-altitude samples. It was increased significantly at high altitude (3.41 ± 1.49 ; $P = 0.013$). Since there was no possibility to obtain cell counts in the high-altitude laboratory, the difference in CS activity allows no conclusion on an altered number mitochondria or increased oxidative activity in the samples obtained at high altitude. $\text{JO}_{2,100}$ was 0.48 ± 0.17 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{unit}^{-1}$ CS at low altitude. Figure 1 shows that $\text{JO}_{2,20}$ at LA was about 40% lower than $\text{JO}_{2,100}$ at LA ($P = 0.002$). $\text{JO}_{2,100}$ at HA was significantly decreased relative to low altitude ($P = 0.018$). Acute hypoxia did not decrease $\text{JO}_{2,20}$ at HA further ($P = 0.241$). Stimulation with PMA increased $\text{JO}_{2,100}$ both at low and high altitude ($P = 0.001$), $\text{JO}_{2,20}$ was increased at high altitude only ($P = 0.001$). There was no difference between $\text{JO}_{2,100}$ at LA and HA ($P = 0.539$) or $\text{JO}_{2,20}$ ($P = 0.469$), respectively, when cells were stimulated with PMA.

Mitochondrial respiration, high-altitude study. Next, we tested whether the decreased oxygen consumption in acute hypoxia during the measurement of JO_2 and the hypoxia at high altitude was due to a decreased mitochondrial activity. Figure 2 summarizes results on complex-I, complex-II, and complex-III respiration. At low altitude, $\text{JO}_{2,C1,20}$ (Fig. 2A) was $\sim 55\%$ lower than $\text{JO}_{2,C1,100}$ ($P = 0.005$), at high altitude, $\text{JO}_{2,C1,20}$ was decreased by $\sim 50\%$ relative to $\text{JO}_{2,C1,100}$ ($P = 0.006$). $\text{JO}_{2,C1,100}$ at HA was significantly lower than at LA ($P = 0.001$), whereas $\text{JO}_{2,C1,20}$ was not different ($P = 0.162$).

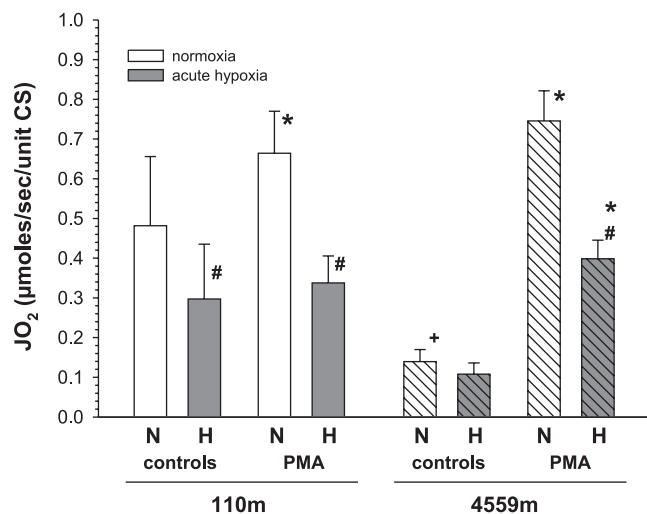


Fig. 1. Effects of acute hypoxia and hypoxia at high altitude (4,559 m) on oxygen consumption (JO_2) of leukocytes. Cells were isolated from whole blood by density gradient centrifugation in Heidelberg (110 m) and after ~20 h in the Capanna Margherita. The medium, which was composed of (in mM): 135 NaCl, 5 KCl, 10 glucose, 1 Na_2HPO_4 , 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 1 mg/ml BSA, pH 7.4 at 37°C, was equilibrated in the respirometer to room air, and leukocytes were added. The oxygen content of the medium was adjusted to a normoxic PO_2 (100 mmHg) by equilibration with the respective gas (see MATERIALS AND METHODS); then the system was closed to record JO_2 . The system was opened again to equilibrate with the hypoxic gas ($PO_2 = 20$ mmHg), and closed again to record JO_2 in acute hypoxia. Measurements were performed in the absence and presence of PMA (200 nM). Values are expressed as means \pm SD from 12 to 15 samples. Significant effect (* $P < 0.05$) of PMA at the respective oxygenation and location; Significant effect (# $P < 0.05$) of acute hypoxia at the respective location and treatment; Significant effect (+ $P < 0.05$) of high altitude.

Also $JO_{2,C2,20}$ (Fig. 2B) was lower than $JO_{2,C2,100}$ at low altitude (-75% ; $P = 0.001$) and at high altitude (-70% ; $P = 0.045$). At high altitude, $JO_{2,C2,100}$ tended to be lower than the low-altitude value $PO_2 = 100$ mmHg ($P = 0.074$), whereas ascent to HA did not affect $JO_{2,C2,20}$ ($P = 0.988$). $JO_{2,C3,100}$ was decreased at high altitude ($P = 0.017$). $JO_{2,C3,20}$ was significantly lower than $JO_{2,C3,100}$ at low altitude (-40% ; $P = 0.004$; Fig. 2C), but there was no statistically significant effect of acute hypoxia at high altitude ($P = 0.557$).

ROS formation, high-altitude study. Because leukocytes actively contribute to respiratory burst, it was important to know whether acute hypoxia and exposure to high altitude affect leukocyte ROS formation. The rate of production of ROS was measured during incubation in the respirometer using the spin probe CMH and ESR spectroscopy for detection of the stable CM^\bullet radical. Figure 3 shows that neither acute hypoxia nor exposure to high altitude significantly affected ROS formation, although ROS formation in normoxia tended to be lower at high altitude ($P = 0.089$). At a PO_2 of 100 mmHg, ROS formation was stimulated significantly with PMA in samples obtained at low and high altitude ($P = 0.001$). When measured at a PO_2 of 20 mmHg, PMA increased ROS formation at high altitude ($P = 0.004$), but there was only a trend toward an increase at low altitude ($P = 0.095$).

In vitro study at low altitude. This series of in vitro experiments was performed to distinguish effects of hypoxia on leukocyte metabolism from possible systemic effects of high altitude. It was also aimed at testing to which extent PMA-

stimulated leukocyte metabolism comes from effects on mitochondria or extra-mitochondrial O_2 -consuming enzymes, such as NADPH oxidases (29), which we could not measure at high altitude. Extra-mitochondrial JO_2 (or residual $JO_{2,res}$) was measured in the absence and presence of rotenone and antimycin A to block complexes I and II. ROS were not measured in this experimental setting, since these inhibitors are known to stimulate ROS production. Figure 4 shows that in normoxia about 20% of total CO - $JO_{2,100}$ were inhibited by rotenone and antimycin A, indicating a significant contribution of $JO_{2,res}$ to

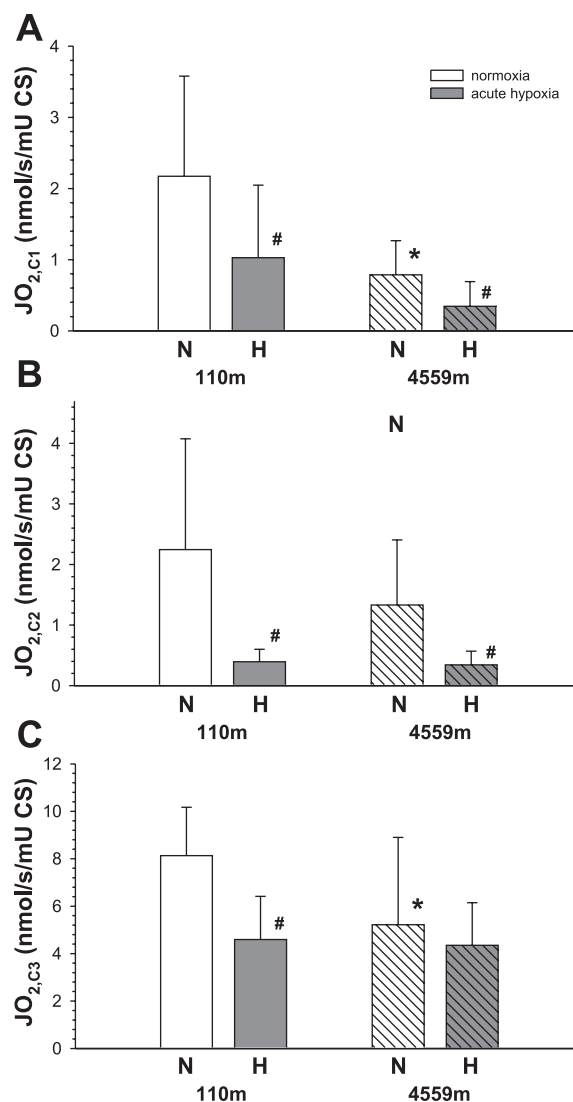


Fig. 2. Effects of acute hypoxia and high-altitude hypoxia on the activity of mitochondrial complexes I, II, and III. Leukocytes were prepared as described in Fig. 1. Mitochondrial oxygen consumption (JO_2) was measured after permeabilizing the cells with digitonin in a medium composed of (in mM) 75 sucrose, 225 mannitol, 10 KCl, 5 KH_2PO_4 , 10 Tris-HCl, 1 mg/ml BSA (fatty acid-free), and 2 malate. State 3 respiration was initiated by adding ADP (2 mM). A: complex I respiration ($JO_{2,C1}$) was measured in presence of 10 mM glutamate. B: complex II respiration ($JO_{2,C2}$) was measured in presence of 10 mM succinate. C: substrate for complex III respiration ($JO_{2,C3}$) was decylubiquinone (50 μ M). Because of the nature of decylubiquinone, sequential measurements were not possible, and separate measurements were performed in normoxia ($PO_2 = 100$ mmHg) and acute hypoxia ($PO_2 = 20$ mmHg). Mean values \pm SE from 12 to 15 samples. #Significant effect ($P < 0.05$) of acute hypoxia at the respective location; +Significant difference ($P < 0.05$) between low and of high altitude.

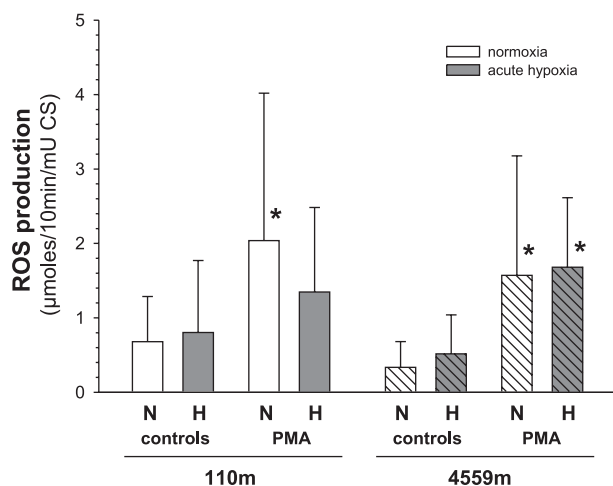


Fig. 3. Effects of acute hypoxia and hypoxia at high altitude (4,559 m) on leukocyte reactive oxygen species (ROS) production. Cells were prepared as for measurement of oxygen consumption (see Fig. 1) in the presence of 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH; 500 μ M) and equilibrated with normoxic and hypoxic gas. Aliquots (250 μ l) were collected at $t = 0$ min, and 10 min later and immediately frozen in liquid nitrogen for CMH measurements by electron spin resonance spectroscopy. Measurements were performed in the absence and presence of 200 nM PMA. N, normoxia ($P_{O_2} = 100$ mmHg); H, hypoxia ($P_{O_2} = 20$ mmHg). Values are expressed as means \pm SD from 15 samples obtained at low altitude (110 m) and after ~ 24 h in the Capanna Regina Margherita (4,559 m). Significant effect ($*P < 0.05$) of PMA at the respective oxygenation and location.

whole cell JO_2 . Acute hypoxia decreased total JO_2 by about 65% ($P < 0.001$), and $JO_{2,res}$ to 20% of the normoxic value ($P < 0.001$). The rotenone + antimycin A inhibitable portion, $JO_{2,m}$ decreased only slightly in acute hypoxia ($P = 0.579$). As expected, PMA significantly increased total JO_2 and $JO_{2,res}$ in normoxia and acute hypoxia. The degree of stimulation of both total JO_2 and extra-mitochondrial JO_2 was significantly lower in acute hypoxia. PMA also increased $JO_{2,m}$ ($P = 0.013$) when it was measured at a $P_{O_2} = 100$ mmHg but not at a $P_{O_2} = 20$ mmHg ($P = 0.178$; Fig. 4).

Figure 4 also shows that neither total JO_2 nor $JO_{2,res}$ and $JO_{2,m}$ measured at $P_{O_2} = 100$ mmHg and 20 mmHg in the absence and presence of PMA were affected significantly by 24-h exposure to hypoxia. The only significant difference to cells exposed to normoxia for 24 h was that $JO_{2,m}$ decreased to barely detectable values in the cells exposed to hypoxia for 24 h when it was measured at a P_{O_2} of 20 mmHg ($P = 0.013$). Also, there was no statistically significant stimulation of $JO_{2,m}$ by PMA in cells that had been exposed to in vitro hypoxia for 24 h ($P = 0.189$).

The mRNA expression of COX-1 and COX-2 was measured as an indicator of possible hypoxia effects on leukocyte gene expression. We found that both COX-1 and COX-2 mRNA were increased significantly by $\sim 50\%$ ($P = 0.035$) after 1 day at 4,559 m. In contrast, there was no statistically significant change in COX-1 and COX-2 in vitro ($P = 0.15$). There was a significant, approximately three-fold increase in the plasma level IL-6 ($P < 0.001$) but no change in TNF- α ($P = 0.784$) (not shown).

DISCUSSION

Hypoxia decreases the activity of oxygen-consuming pathways to conserve energy and to prevent cell death (37).

However, it also decreases cellular respiration (37), which might impair cellular functions. Here, we studied whether leukocyte oxidative metabolism and the respiratory burst were affected by hypoxia in vivo and in vitro to separate direct hypoxia effects from systemic influences. Direct effects of hypoxia were studied by measuring cellular respiration in media equilibrated to hypoxic gas mixtures (total exposure time: ~ 15 min) and after culturing of leukocytes from nor-

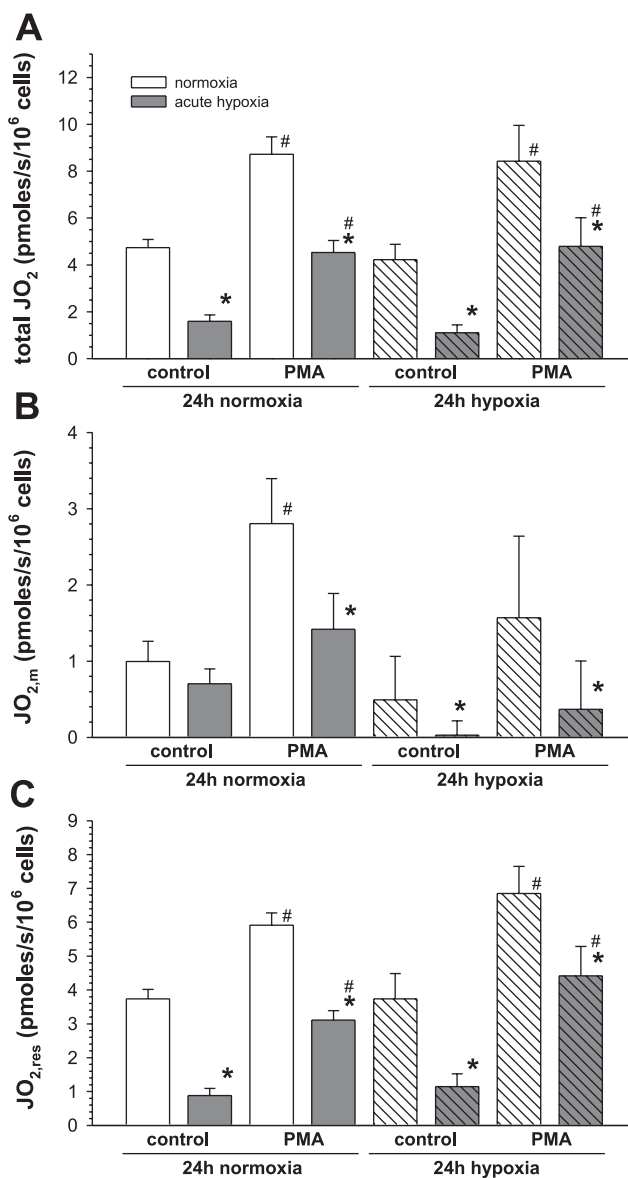


Fig. 4. Effects of 24 h hypoxia and PMA on mitochondrial and residual oxygen consumption. Human leukocytes prepared from whole blood of normoxic individuals ($n = 6$) by density gradient centrifugation were cultured in normoxia and hypoxia (1.5% O_2) for 24 h. Oxygen consumption was measured in normoxia ($P_{O_2} \sim 100$ mmHg) and in acute hypoxia ($P_{O_2} \sim 20$ mmHg) in the absence and presence of 200 nmol PMA. A: total leukocyte oxygen consumption, $JO_{2,tot}$. B: mitochondrial oxygen consumption ($JO_{2,m}$) was determined as the proportion of JO_2 inhibited by rotenone (0.5 μ M) and antimycin A (2.5 μ M). C: residual oxygen consumption ($JO_{2,res}$) is the JO_2 measured in presence of rotenone and antimycin A; it serves as a measure of O_2 consumed by oxidases such as NADPH oxidase. Mean values \pm SD from measurements on six independent samples. Significant effect ($\#P < 0.05$) of PMA. Significant effect ($*P < 0.05$) of acute hypoxia.

moxic individuals for 24 h in hypoxia. To test for systemic effects, measurements were performed on leukocytes from individuals exposed to high altitude. The major finding of this study was that acute *in vitro* hypoxia decreased oxygen consumption. Oxygen consumption was not different between cells cultured in normoxia or hypoxia for 24 h, regardless of the P_{O_2} at which it was measured. In contrast, oxygen consumption of leukocytes from individuals exposed to *in vivo* hypoxia at high altitude (4,559 m) was lower than prealtitude values when measured at normoxic P_{O_2} . This was paralleled by a decreased activity of complexes I, II, and III of the mitochondrial electron transfer chain. There was no difference between low and high altitude in $JO_{2,20}$. Respiratory burst induced with PMA was decreased by acute hypoxia but was not affected by 24 h of hypoxia when measured at a defined P_{O_2} . These results indicate that during high-altitude exposure, oxygen consumption of leukocytes was decreased by hypoxia directly but also by systemic effects. Decreased oxygen consumption is paralleled by lower mitochondrial respiration and by decreased respiratory burst.

Leukocyte metabolism in acute hypoxia. Acute hypoxia has been shown to inhibit cellular O_2 consumption by decreasing mitochondrial respiration in a variety of cells (see, e.g., 21, 22, 39). Here, we show that in blood samples obtained from normoxic individuals acute *in vitro* hypoxia ($P_{O_2} = 20$ mmHg) applied during the measurements of oxygen consumption significantly decreased basal leukocyte oxygen consumption relative to values obtained in simulated normoxia at a P_{O_2} of 100 mmHg. A decrease in JO_2 has been interpreted to reflect a decreased energy demand upon reducing the activity of ATP-consuming processes, such as Na/K-ATPase and protein synthesis (22). Similar to alveolar epithelial cells (21), we show here that in leukocytes the decrease in basal JO_2 was paralleled by a decrease in the capacity of complexes I and II of the mitochondrial electron transfer chain (mETC). Thus, in hypoxia, basal leukocyte oxygen consumption is decreased by a decrease in mitochondrial respiration.

Leukocytes are capable of respiratory burst that depends on the activity of NADPH oxidase. Since this enzyme directly uses O_2 to form $O_2^{\bullet-}$ (for review, see Ref. 18), it is conceivable that hypoxia also decreases extramitochondrial oxygen consumption by a limitation of substrate (25). We show here in accordance with Kozlov et al. (26) that stimulation of leukocytes with PMA increases ROS production and total JO_2 . Our results shown in Fig. 4 indicate that in nonstimulated leukocytes extra-mitochondrial oxygen consumption amounts to about 70% of total JO_2 , that PMA induced respiratory burst causes an increase in JO_2 , and that more than 80% of JO_2 is extra-mitochondrial upon stimulation with PMA. In support of our hypothesis, we show here that both basal and PMA-stimulated extra-mitochondrial oxygen consumption was decreased by acute hypoxia. This supports the notion that a decrease in P_{O_2} also decreases oxidase activity (14).

On the basis of the above described results and those obtained by Gabig et al. (14), a decreased leukocyte ROS formation can also be expected in hypoxia, which should indicate impaired oxidative burst (8, 14). Surprisingly, neither basal nor PMA-stimulated ROS formation was significantly affected by acute exposure to hypoxia. A likely explanation might be an inverse change in mitochondrial and NADPH oxidase-dependent ROS formation. An increased ROS forma-

tion by the mETC has been found in various cell types when the P_{O_2} was very low (4, 6). Complexes II (31) and III (5) of the mETC have been discussed as possible sites of $O_2^{\bullet-}$ formation in hypoxia. In contrast, ROS formation by NADPH oxidase is decreased when the P_{O_2} is very low since it requires oxygen as a substrate. Thus, an unchanged rate of ROS formation might be the result of a decreased activity of NADPH oxidase and increased ROS formation in the mETC. This assumption implies that the relative contribution of mitochondrial and extra-mitochondrial ROS production determines the rate of ROS production measured in a specific cell at a given P_{O_2} , where the relative amounts or activities of NADPH oxidase and mitochondria is specific for a certain cell type. This might explain the discrepant results on ROS formation observed in hypoxia (e.g., 1, 35).

Effects of prolonged in vitro hypoxia. *In vitro* hypoxia for 24 h of leukocytes collected from normoxic donors did not affect total JO_2 , regardless of the P_{O_2} at which JO_2 was measured. It tended to decrease mitochondrial JO_2 measured as the proportion of JO_2 inhibited by rotenone and antimycin A, whereas extra-mitochondrial JO_2 was not affected. As in normoxic cells, acute hypoxia applied in the respirometer significantly decreased total JO_2 also in the cells exposed to hypoxia for 24 h, whereas mitochondrial respiration dropped to barely detectable values (Fig. 4). These results indicate that 24h of *in vitro* hypoxia seems not to impair leukocyte metabolism when measured at normoxic P_{O_2} but caused a pronounced decrease in mitochondrial JO_2 when cells are acutely hypoxic.

PMA increased total as well as mitochondrial JO_2 of hypoxic cells by the same degree as in normoxic ones, indicating that prolonged hypoxia of leukocytes did not affect the capacity of respiratory burst at a given P_{O_2} . PMA-stimulated JO_2 was lower in cells exposed to 24 h of *in vitro* hypoxia when measured at a P_{O_2} of 20 mmHg rather than at 100 mmHg, indicating that acute hypoxia impaired respiratory burst. We did not measure ROS production under these conditions since inhibition of complex I and III by treatment with rotenone and antimycin A, respectively, increases ROS production, which might affect other responses. However, results from separate test experiments (not shown) indicate parallel changes of ROS and JO_2 , indicating that extra-mitochondrial JO_2 is a suitable indicator of ROS formation. Taken together, these results indicate that *in vitro* hypoxia for 24 h seems not to impair cellular respiration and NADPH oxidase activity when measured at high P_{O_2} , whereas at low P_{O_2} , both components are decreased.

Effects of exposure to high altitude. It has been shown that the cell-proliferative activity and expression of proinflammatory cytokines were decreased after slow ascent and a 3-wk stay at high altitude (12), which is indicative of impaired leukocyte function during prolonged *in vivo* hypoxia (12). Further studies are needed, however, to discriminate whether these changes are due to hypoxia alone or whether systemic effects of hypoxia and the exercise during ascent also affected leukocytes. Exercise has been shown to stimulate immune-competent cells as indicated by elevated expression of heat shock proteins (13), but it has also been shown to be immunosuppressive by decreasing leukocyte counts and function (15). Meehan et al. (28) reported a decrease in protein synthesis and thymidine uptake in mononuclear cells from blood samples obtained from individuals exposed to simulated extreme alti-

tude during Operation Everest II. Here, we showed that the basal oxygen consumption was decreased in leukocytes collected from individuals ~24 h after reaching an altitude of 4,559 m, which was paralleled by a decrease in the activity of mETC-complexes I and III when measured at a P_{O_2} of 100 mmHg. Acute hypoxia of the leukocytes obtained at high altitude did not decrease total JO_2 but decreased complexes I and II. However, values were not different from those obtained in low-altitude cells acutely exposed to hypoxia. Basal ROS formation tended to decrease at high altitude, but PMA-stimulated ROS formation was not affected, regardless of the P_{O_2} at which it had been measured. This is also indicated by an unchanged oxygen consumption of PMA-stimulated leukocytes. It is unclear to what extent NADPH oxidase and other cytosolic oxidases might contribute to this result since no measure of extra-mitochondrial oxygen consumption could be obtained in the high-altitude study. These results indicate that cellular respiration of leukocytes was decreased after ascent to high altitude, whereas the capacity of respiratory burst was not impaired.

Recent studies suggest that hypoxia might initiate a systemic proinflammatory process by mediators released from lung alveolar macrophages (17) resulting in leukocyte activation (42). This contrasts the observations described above. Our results on elevated mRNA expression of COX-1 and COX-2 in leukocytes collected at high altitude are also indicative of leukocyte stimulation. However, since COX expression was not altered by in vitro hypoxia, it appears that the increased COX expression is an effect secondary to the in vivo hypoxia at high altitude and, possibly, the exercise during ascent, but not a direct effect of the hypoxia.

The mechanisms causing the changes in leukocyte metabolism upon ascent to high altitude are not clear. Increased plasma levels of catecholamines at high altitude and with exertion (for review, see Ref. 3) might play a role since they are known to impair leukocyte function (34). Decreased mitochondrial function might be a consequence of hypoxia-induced autophagy initiated by HIF-1 α , a well-known mechanism protecting cells from damage by elevated mitochondrial ROS production during severe hypoxia (37). However, in an earlier study, at the same altitude and with the same profile of ascent, we found no indications for stimulation of HIF-1 α in leukocytes indicated by unchanged mRNA expression of the glycolytic enzyme GAPD (27), whose expression is up-regulated when HIF-1 α is increased. This might indicate that, for leukocytes, the degree of hypoxia at this altitude is insufficient to increase HIF-1 α activity (27) and that the changes observed are independent of HIF-1 α .

In conclusion, we show here that acute in vitro hypoxia decreased leukocyte metabolic activity and mitochondrial respiration. Thus, it can be speculated that leukocyte metabolism is decreased at high altitude when the P_{O_2} of blood is decreased. The fact that respiration of leukocytes was not affected by in vitro 24-h hypoxia but was decreased in leukocytes from individuals at high altitude when measured at normoxic P_{O_2} might indicate that systemic factors also appear to affect leukocyte metabolism and the respiratory burst in high-altitude hypoxia. This notion is further supported by our results showing that the mRNA expression of COX-1 and COX-2 in leukocytes was increased at high altitude but not by in vitro hypoxia. We cannot discriminate whether the exercise during

ascent or systemic effects of hypoxia at high altitude itself caused this effect. PMA-stimulated oxygen consumption, a measure of the capacity of respiratory burst, and leukocyte ROS formation were not impaired in leukocytes from high altitude when measured at normoxic P_{O_2} . In contrast, acute hypoxia decreased respiratory burst. Thus, our results indicate that acute, severe hypoxia impairs leukocyte function at low and at high altitude.

ACKNOWLEDGMENTS

We are most grateful to the subjects participating in this study, as well as to the hut keepers and the Sezione Varallo of the Club Alpino Italiano for providing an excellent research facility at the Capanna Regina Margherita. The expert technical assistance of Mrs. Sonja Engelhardt and Mrs. Christiane Herth is gratefully acknowledged.

GRANTS

This study was supported in part by a grant from the German Research Foundation Grant Ma 1503/14-1 (to H. Mairbäurl.).

DISCLOSURES

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

REFERENCES

1. Acker H. The oxygen sensing signal cascade under the influence of reactive oxygen species. *Philos Trans R Soc Lond B Biol Sci* 360: 2201–2210, 2005.
2. Allen DB, Maguire JJ, Mahdavian M, Wicke C, Marcocci L, Scheuenstuhl H, Chang M, Le AX, Hopf HW, Hunt TK. Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. *Arch Surg* 132: 991–996, 1997.
3. Blume FD. Metabolic and endocrine changes at altitude. In: *High Altitude and Man*, edited by West JB and Lahiri S. Baltimore, MD: Williams and Wilkins, 1984, p. 37–45.
4. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci USA* 95: 11715–11720, 1998.
5. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, Schumacker PT. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1 α during hypoxia—A mechanism of O_2 sensing. *J Biol Chem* 275: 25130–25138, 2000.
6. Dada LA, Chandel NS, Ridge KM, Pedemonte C, Bertorello AM, Sznajder JL. Hypoxia-induced endocytosis of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC- ζ . *J Clin Invest* 111: 1057–1064, 2003.
7. Dehnert C, Luks AM, Schendler G, Menold E, Berger MM, Mairbäurl H, Faoro V, Bailey DM, Castell C, Hahn G, Vock P, Swenson ER, Bartsch P. No evidence for interstitial lung oedema by extensive pulmonary function testing at 4559 m. *Eur Respir J* 35: 812–820, 2010.
8. Derevianko A, Graeber T, D'Amico R, Simms H. Altered oxygen tension modulates cytokine-induced signal transduction in polymorphonuclear leukocytes: regulation of the G PLC pathway. *J Surg Res* 62: 32–40, 1996.
9. Di Lisa F, Bernardi P. Mitochondrial function as a determinant of recovery or death in cell response to injury. *Mol Cell Biochem* 184: 379–391, 1998.
10. Dikalov S, Fink B, Skatchkov M, Sommer O, Bassenge E. Formation of reactive oxygen species in various vascular cells during glyceryltrinitrate metabolism. *J Cardiovasc Pharmacol Ther* 3: 51–62, 1998.
11. Ehleben W, Bolling B, Merten E, Porwol T, Strohmaier AR, Acker H. Cytochromes and oxygen radicals as putative members of the oxygen sensing pathway. *Respir Physiol* 114: 25–36, 1998.
12. Facco M, Zilli C, Siviero M, Ermolao A, Travain G, Baesso I, Bonamico S, Cabrelle A, Zaccaria M, Agostini C. Modulation of immune response by the acute and chronic exposure to high altitude. *Med Sci Sports Exerc* 37: 768–774, 2005.
13. Fehrenbach E, Niess AM, Schlotz E, Passek F, Dickhuth HH, Northoff H. Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J Appl Physiol* 89: 704–710, 2000.

14. **Gabig TG, Bearman SI, Babior BM.** Effects of oxygen tension and pH on the respiratory burst of human neutrophils. *Blood* 53: 1133–1139, 1979.
15. **Gani F, Passalacqua G, Senna G, Mosca FM.** Sport, immune system and respiratory infections. *Eur Ann Allergy Clin Immunol* 35: 41–46, 2003.
16. **Gnaiger E, Kuznetsov AV.** Mitochondrial respiration at low levels of oxygen and cytochrome *c*. *Biochem Soc Transact* 30: 252–257, 2002.
17. **Gonzalez NC, Allen J, Blanco VG, Schmidt EJ, van Rooijen N, Wood JG.** Alveolar macrophages are necessary for the systemic inflammation of acute alveolar hypoxia. *J Appl Physiol* 103: 1386–1394, 2007.
18. **Halliwell B, Gutteridge JMC.** *Free Radicals In Biology and Medicine*. Oxford, UK: Oxford University Press, 2002.
19. **Hannah S, Mecklenburgh K, Rahman I, Bellingan GJ, Greening A, Haslett C, Chilvers ER.** Hypoxia prolongs neutrophil survival in vitro. *FEBS Lett* 372: 233–237, 1995.
20. **Harris SI, Patton L, Barrett L, Mandel LJ.** (Na⁺,K⁺)-ATPase kinetics within the intact renal cell. The role of oxidative metabolism. *J Biol Chem* 257: 6996–7002, 1982.
21. **Heerlein K, Schulze A, Hotz L, Bärtsch P, Mairbäurl H.** Hypoxia decreases cellular ATP-demand and inhibits mitochondrial respiration of A549 cells. *Am J Respir Cell Mol Biol* 32: 44–51, 2005.
22. **Hochachka PW.** Defense strategies against hypoxia and hypothermia. *Science* 231: 234–241, 1986.
23. **Hood DA, Zak R, Pette D.** Chronic stimulation of rat skeletal muscle induces coordinate increases in mitochondrial and nuclear mRNAs of cytochrome-*c*-oxidase subunits. *Eur J Biochem* 179: 275–280, 1989.
24. **Jarstrand C, Wiernik A, Revesz L.** Significance of oxygen availability for release of oxygen free radicals and lysozyme by neutrophils. *J Clin Lab Immunol* 32: 37–39, 1990.
25. **Jones RD, Hancock JT, Morice AH.** NADPH oxidase: a universal oxygen sensor? *Free Radic Biol Med* 29: 416–424, 2000.
26. **Kozlov AV, Szalay L, Umar F, Fink B, Kropik K, Nohl H, Redl H, Bahrami S.** Epr analysis reveals three tissues responding to endotoxin by increased formation of reactive oxygen and nitrogen species. *Free Radic Biol Med* 34: 1555–1562, 2003.
27. **Mairbäurl H, Schwöbel F, Höschele S, Maggiorini M, Gibbs JSR, Swenson E, Bärtsch P.** Altered expression of ion transporters in bronchial epithelium and in leukocytes of mountaineers suffering from HAPE at 4559 m. *J Appl Physiol* 95: 1843–1850, 2003.
28. **Meehan R, Duncan U, Neale L, Taylor G, Muchmore H, Scott N, Ramsey K, Smith E, Rock P, Goldblum R.** Operation Everest II: alterations in the immune system at high altitudes. *J Clin Immunol* 8: 397–406, 1988.
29. **Morel F, Doussiere J, Vignais PV.** The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. *Eur J Biochem* 201: 523–546, 1991.
30. **Moudgil R, Michelakis ED, Archer SL.** Hypoxic pulmonary vasoconstriction. *J Appl Physiol* 98: 390–403, 2005.
31. **Paddenberg R, Ishaq B, Goldenberg A, Faulhammer P, Rose F, Weissmann N, Braun-Dullaeus RC, Kummer W.** Essential role of complex II of the respiratory chain in hypoxia-induced ROS generation in the pulmonary vasculature. *Am J Physiol Lung Cell Mol Physiol* 284: L710–L719, 2003.
32. **Roach RC, Bärtsch P, Hackett PH, Oelz O.** The Lake Louise acute mountain sickness scoring system. In: *Hypoxia and Molecular Medicine*, edited by Sutton JR, Houston CS, and Coates G. Burlington, VT: Quenn City Printers, 1993, p. 272–274.
33. **Sampson JB, Cymerman A, Burse RJ, Maher JT, Rock PB.** Procedures for the measurement of acute mountain sickness. *Aviat Space Environ Med* 54: 1063–1073, 1983.
34. **Schopf RE, Lemmel EM.** Control of the production of oxygen intermediates of human polymorphonuclear leukocytes and monocytes by beta-adrenergic receptors. *J Immunopharmacol* 5: 203–216, 1983.
35. **Schumacker PT.** Hypoxia, anoxia, and O₂ sensing: the search continues. *Am J Physiol Lung Cell Mol Physiol* 283: L918–L921, 2002.
36. **Schumacker PT, Chandel N, Agusti AGN.** Oxygen conformance of cellular respiration in hepatocytes. *Am J Physiol Lung Cell Mol Physiol* 265: L395–L402, 1993.
37. **Semenza GL.** Mitochondrial autophagy: life and breath of the cell. *Autophagy* 4: 534–536, 2008.
38. **Semenza GL, Roth PH, Fang HM, Wang GL.** Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 269: 23757–23763, 1994.
39. **Storey KB, Storey JM.** Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Q Rev Biol* 65: 145–174, 1990.
40. **Taylor DE, Kantrow SP, Piantadosi CA.** Mitochondrial respiration after sepsis and prolonged hypoxia. *Am J Physiol Lung Cell Mol Physiol* 275: L139–L144, 1998.
41. **Walmsley SR, Print C, Farahi N, Peyssonnaud C, Johnson RS, Cramer T, Sobolewski A, Condliffe AM, Cowburn AS, Johnson N, Chilvers ER.** Hypoxia-induced neutrophil survival is mediated by HIF-1 α -dependent NF- κ B activity. *J Exp Med* 201: 105–115, 2005.
42. **Wood JG, Johnson JS, Mattioli LF, Gonzalez NC.** Systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats. *J Appl Physiol* 89: 1561–1568, 2000.
43. **Wyche KE, Wang SS, Griendling KK, Dikalov SI, Austin H, Rao S, Fink B, Harrison DG, Zafari AM.** C242T CYBA polymorphism of the NADPH oxidase is associated with reduced respiratory burst in human neutrophils. *Hypertension* 43: 1246–1251, 2004.