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Acute Hypoxemia Does Not Increase the Oxidative Stress in Resting and Contracting Muscle in Humans

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In healthy humans sustaining static handgrip at 60% of maximal voluntary contraction (MVC) until exhaustion, we measured the venous blood concentration of reduced ascorbic acid (RAA) and thiobarbituric acid reactive substances (TBARS), respectively, used as markers of the post-exercise oxidative stress and lipid peroxidation. Measurements were conducted in normoxemia, then during a 30-min period of hypoxemia ($PaO_2 = 56$ mmHg) produced by inhalation of an hypoxic gas mixture. Compared to normoxemia, hypoxemia did not significantly modify the resting concentrations of TBARS and RAA, and did not affect the consumption of ascorbic acid after 60% MVC but suppressed the post-exercise TBARS increase. We conclude that acute hypoxemia does not modify the production of oxygen free radicals after strenuous static efforts and even seems to attenuate the lipid peroxidation.

Keywords: Static contraction; Handgrip; Oxidative stress; Hypoxemia

INTRODUCTION

An oxidative stress due to enhanced production of oxygen free radicals is associated with strenuous exercise in humans[1–8] and animals.[9,10] The post-exercise oxidative stress is explored through measurements of blood markers, which indicate either the formation of lipid hydroperoxides, as the thiobarbituric acid reactive substances (TBARS),[4,8,11] and the consumption of endogenous antioxidants.[11–15] In animals[16] and humans,[11] chronic hypoxemia (lowered PaO_2 level) accentuates the lipid peroxidation in numerous organs including muscle. However, conflicting data are found in the literature on the consequences of acute hypoxia on the formation of reactive oxygen species (ROS). In humans, acute hypobaric hypoxia promotes an oxidative stress condition in the erythrocyte membrane[17] whereas the ROS production by rat heart mitochondria is reduced during acute hypoxia[18] and the generation of ROS by bovine endothelial cells is markedly attenuated during anoxia.[19]

We questioned if a brief (30 min) period of hypoxemia modifies the post-exercise oxidative stress in humans. To test this hypothesis we measured the plasma concentrations of TBARS and reduced ascorbic acid (RAA) in healthy volunteers at rest and after a static handgrip sustained at 60% of maximal voluntary contraction (MVC) until exhaustion.

MATERIALS AND METHODS

Eight male subjects (mean age: 30 ± 3 years; weight: 76 ± 4 kg) participated in the study. The Local Ethics Committee approved the protocol. On the side of the working forearm muscle, 5 ml of heparinized blood were sampled from an antecubital vein at each

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TABLE I Experimental conditions. Measurements of partial pressures of oxygen, carbon dioxide and pH in arterialized blood at rest and before the end of 60% MVC handgrip exercise. Asterisks indicated significant differences between data measured at rest and during handgrip (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Crosses shows significant differences between normoxemic and hypoxemic conditions (+: $p < 0.05$, +++: $p < 0.001$). No significant differences were noted between the left and right arms.

	Normoxemia		Hypoxemia	
	Rest	Handgrip	Rest	Handgrip
		PaO_2		
Right arm	90 ± 2	109 ± 7*	59 ± 3^{+++}	68 ± 2*$^{+++}$
Left arm	94 ± 2	98 ± 4	61 ± 3^{+++}	68 ± 2^{+++}
Mean	92 ± 2	104 ± 3*	60 ± 2^{+++}	68 ± 1***$^{+++}$
		$PaCO_2$		
Right arm	40 ± 1	33 ± 2*	32 ± 3^{+}	29 ± 3*
Left arm	38 ± 2	34 ± 2	32 ± 4	27 ± 4*
Mean	39 ± 1	34 ± 1**	32 ± 2^{++}	28 ± 2^{+}***
		pHa		
Right arm	7.40 ± 0.01	7.45 ± 0.01	7.46 ± 0.03^{+++}	7.50 ± 0.07
Left arm	7.40 ± 0.01	7.44 ± 0.01	7.47 ± 0.04	7.5 ± 0.04**
Mean	7.40 ± 0.01	7.44 ± 0.01**	7.47 ± 0.02	7.50 ± 0.02^{+}***

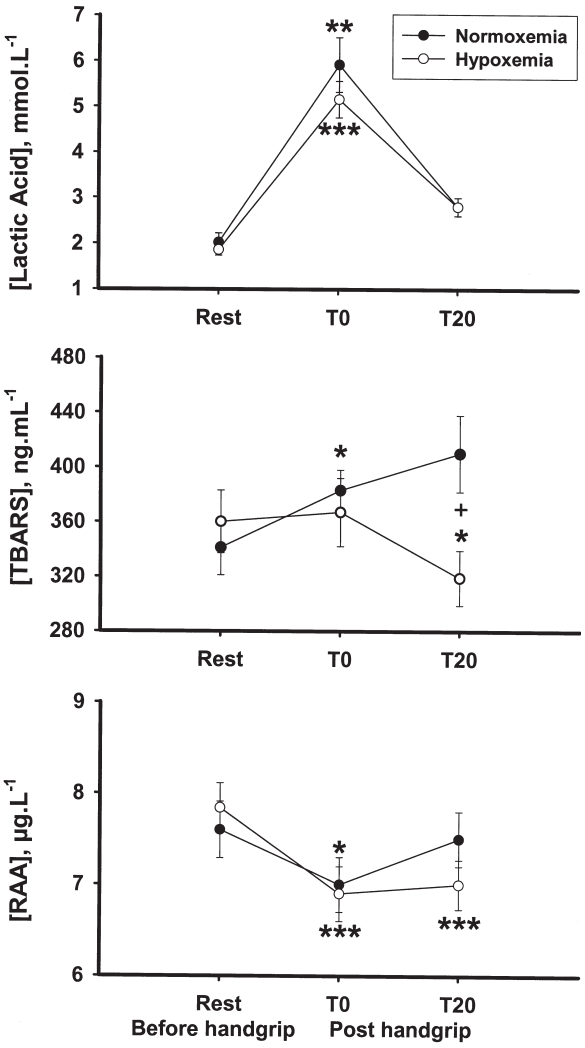

FIGURE 1 Plasma concentrations of lactic acid, TBARS, and RAA in resting forearm, immediately after the 60% MVC handgrip ended (*T*0), and at 20 min of recovery (*T*20). This was studied in normoxemia, then hypoxemia. Values are mean ± SEM. Asterisks indicate that values differ significantly from resting ones in each situation (∗$p < 0.05$; ∗∗ $p < 0.01$; ∗∗∗$p < 0.001$).

sequence of the protocol. Lactic acid was estimated with the enzymatic method (Bayer Diagnostics model 860, Paris, France) and the plasma TBARS concentration was measured using a spectrofluorimeter (SHIMADZU model RF 5000, Kyoto, Japan) according to the method by Uchiyama and Mihara.[20] RAA was estimated by spectrophotometry using the method based on the reduction of iron by ascorbic acid in the presence of orthophosphoric acid and α-α-dipyridyl.[21] Prior to the experiment, the ear lobe was pretreated with an active vasodilator cream. Then, it was incised to sample arterialized blood in 100 µl heparinized capillary tubes. Arterial blood gases (PaO_2, $PaCO_2$ and pHa) were analyzed (model 860 Bayer Diagnostics, Puteaux, France). The subject was comfortably seated. One forearm was maintained in prone position in an anatomic device that allowed isometric handgrip.[22] The force was measured with a strain gauge (Scaime model ZF, Annemasse, France), linear from 0 to 100 kg. The subject performed three 5-s isometric MVCs, with a 2 min interval between each contraction, the highest force being considered the MVC. After a 10-min rest period, the subject was asked to sustain handgrip at 60% MVC with the aid of the reference value indicated on the strain gauge amplifier. When torque had leveled off close to the preset value (endurance time), the subject ceased contraction and kept rest for a consecutive 20-min period. Venous blood was sampled prior to the beginning of the 60% MVC (rest value), immediately after contraction had stopped (*T*0), then at 20 min (*T*20) during the recovery period.

Hypoxemia was produced by inhaling a mixture of 15% oxygen in nitrogen and the handgrip trial began after a 10-min period of stable hypoxemia. The magnitude of sustained handgrips was the same in hypoxemic condition, i.e. 60% of MVC measured in normoxemia.

In all subjects, arterialized blood was sampled at the end of the period of normoxemia ($PaO_2 =$ 88 mmHg), then, at 10 and 30 min during the period of hypoxemia. No significant difference in PaO_2 was found in resting subjects at 10 and 30 min of hypoxemia (mean $PaO_2 = 56 \pm 1$ mmHg). In four subjects, blood gases were also analyzed close to the endurance time to sustain 60% MVC in normoxemia and hypoxemia in order to estimate the consequences of exercise-induced ventilatory changes on PaO_2, $PaCO_2$, and pHa. As shown in Table I, PaO_2 significantly increased at the end of handgrip trial in normoxemia (+12 mmHg, $p < 0.05$) as well as in hypoxemia (+8 mmHg, $p < 0.001$), but hypoxemia persisted throughout the exercise period. No significant differences were found between the two arms.

The whole challenge (normoxemia and hypoxemia) was studied in the contralateral forearm on a separate day, and it was also repeated in the dominant forearm of individuals. Thus, 25 complete challenges were studied in the eight subjects.

An analysis of variance with repeated measures was used to determine whether significant differences existed between pre- and post-fatigue values of lactic acid, TBARS and RAA concentrations in the two situations (normoxemia and hypoxemia). A difference was accepted as statistically significant if $p < 0.05$. Data are presented as means ± SE.

RESULTS

The endurance time to sustain 60% MVC did not significantly differ in normoxemia (42 ± 5 s) and hypoxemia (49 ± 6 s). Thus, the magnitude and duration of static exercises were the same in the two situations.

In normoxemia as well as hypoxemia, there was no significant difference between venous blood concentrations of lactic acid, TBARS and RAA measured in the right and left forearms at rest as well as after 60% MVCs. Thus, data are a mean of the 25 challenges performed in normoxemia then hypoxemia.

As shown in Fig. 1, the 60% MVC performed in normoxemia was followed by a significant increase in concentration of lactic acid ($p < 0.01$) and TBARS ($p < 0.05$), whereas the RAA concentration decreased ($p < 0.05$). Compared to values measured in normoxemia, hypoxemia did not significantly affect resting value of lactic acid, TBARS, and RAA nor the maximal post-exercise changes in lactic acid and RAA. However, hypoxemia suppressed the post-exercise increase in TBARS, which was even replaced by a significant decrease at *T*20 ($p < 0.05$).

DISCUSSION

Blood gases analyses revealed that the subjects continued to breathe during 60% MVC sustained until exhaustion. Thus, the post-exercise oxidative stress reported in our experiment solely resulted from the strenuous work and not from the consequences of a brief period of asphyxia due to hypoventilation or apnea. In addition, hypoxemia was near stable during the whole challenge, including the period of handgrip exercises.

As previously reported in numerous human studies,[23] we confirm that acute hypoxemia does not increase the resting blood lactic acid concentration. It is well known that hypoxemia increases the lactic acid production during lengthened dynamic muscle contractions,[23] but there are no data in the literature on similar effects in the condition of static contractions sustained for very limited periods.

In this study we measured TBARS concentration to estimate the magnitude of lipid peroxidation. Measurements of plasma concentration of TBARS and malonaldehyde have been widely used to estimate lipid peroxidation.[1,5,7,14] However, TBARS must be considered as an indirect method and Alessio[5] in her review has insisted on the necessity to associate this method with measurements of endogenous antioxidants, as the RAA.

Compared to normoxemia, acute hypoxemia suppressed the TBARS increase after sustained handgrip, attenuating lipid peroxidation in exercising muscle. Sjödin *et al*.[2] have already hypothesized that an abrupt marked reduction of oxygen supply to muscle should decrease the formation of oxygen free radicals through the reduction of metabolic aerobic pathways and myoglobin oxygen stores. Our data did not entirely corroborate this hypothesis because acute hypoxemia did not affect the resting values of TBARS and RAA, and also did not change the post-exercise RAA decrease.

In experimental situations of prolonged chronic hypoxemia in animals[16] and humans[11] resting values of TBARS increased in the circulating blood[11] and also in different organs (skeletal muscle, heart, liver and lungs),[16] but no significant variations of baseline concentrations of RAA and reduced glutathione were reported.[11] Although the PaO_2 value measured at high simulated altitude[11] was the same than that reached in the present study, chronic hypoxemia significantly increased the rise of TBARS and the reduction of RAA and reduced glutathione measured after cycling exercise. The major discordances between the consequences of acute and chronic hypoxemia on the post-exercise oxidative stress are the dissociated variations of TBARS and endogenous antioxidants. We found data in the literature on facilitating effects of acute

hypoxemia on the glucose transport through the cell membrane, and into the cytoplasm and also on its binding with specific membrane receptors.[24] In addition, hypoxemia prevents phospholipid degradation in ventricular myocytes by reducing their phospholipase A activities[25] and enhances the expression of plasma phospholipid transfer protein.[26] These informations suggest that the organism seems to develop some defense mechanisms to protect the cell membrane against acute hypoxemia. This hypothesis implies that these mechanisms could be investigated in skeletal muscle.

The present observations suggest that there are no risks of enhanced post-exercise oxidative stress during a short period of reduced oxygen supply to muscle. However, further studies are needed to explore the consequences of acute hypoxemia on the antioxidant–oxidant status during dynamic exercise. Indeed, static muscular contractions at a high strength interrupt muscle blood flow in both normoxemic and hypoxemic conditions. By contrast, muscle blood flow markedly increases during dynamic exercise and a moderate hypoxemia accentuates this phenomenon.[27] Blood flow changes being responsible for marked variations of oxygen and energetic supplies to muscle, this may greatly affect the cellular formation of oxygen free radicals.

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