Original Research

Gradually increased oxygen administration promoted survival after hemorrhagic shock

Xin Luo, Gan Chen, Guoxing You, Bo Wang, Mingzi Lu, Jingxiang Zhao, Ying Wang, Yujing Yin, Lian Zhao and Hong Zhou

Department of Blood Products and Substitutes, Institute of Transfusion Medicine, Academy of Military Medical Sciences, Beijing 100850, PR China

Corresponding authors: Yujing Yin. Email: yyjhbw@163.com; Lian Zhao. Email: zhaolian@bmi.ac.cn; Hong Zhou. Email: zhouhtt1966@163.com

Abstract

Gradually increased oxygen administration (GIOA) seems promising in hemorrhagic shock. However, the effects of GIOA on survival remain unclear, and details of GIOA are to be identified. After the induction of hemorrhagic shock, the rats were randomized into five groups ($n = 9$): normoxic group (Normo), hyperoxic group (Hypero), normoxic to hyperoxic group (GIOA1), long-time hypoxemic to hyperoxic group (GIOA2), and short-time hypoxemic to hyperoxic group (GIOA3). Survival was recorded for 96 h, plasma alanine transaminase, oxidative stress, hemodynamics, and blood gas were measured. The mean survival time of the GIOA3 was significantly longer than that of the Normo, Hypero, and GIOA2. Plasma alanine transaminase levels were significantly lower in the Normo, GIOA1, and GIOA3 compared to the Hypero and GIOA2 at 2 h post-resuscitation (PR). Plasma 3-nitrotyrosine levels at 2 h PR were significantly lower in the GIOA2 and GIOA3 compared to the Normo and Hypero. Central venous oxygen saturation at 2 h PR in the GIOA3 was significantly higher than the Normo; however, no significant difference was observed between GIOA1 and Normo. Besides, at 2 h PR, mean arterial pressure in the GIOA3 was significantly higher than the GIOA2; however, no significant difference was observed between GIOA1 and GIOA2. (1) GIOA could significantly prolong survival time compared to normoxemic resuscitation and hyperoxic resuscitation; (2) early moments of GIOA are critical to the benefits; and (3) hypoxemia at onset of resuscitation may be imperative, more works are needed to determine the optimal initial oxygen concentration of GIOA.

Keywords: Hemorrhagic shock, resuscitation, hyperoxic, normoxic, hypoxemic, gradual treatment

***Experimental Biology and Medicine* 2016; 241: 1603–1610. DOI: 10.1177/1535370216644996**

Introduction

Hemorrhagic shock (HS) is one of the major causes of death in trauma. HS impairs oxygen delivery, causes cellular and tissue hypoxia, and may ultimately result in multiple organ dysfunction syndrome (MODS).[1,2] To restore tissue and systemic oxygenation as soon as possible, hyperoxic resuscitation has been the routine method during clinical HS management.[3–5] However, the potential side effects of hyperoxic resuscitation could offset the benefits, too much oxygen may be associated with vasoconstriction and the oxidative burst.[6–8] To alleviate oxidative injury during resuscitation from HS, hypoxemic resuscitation was proposed.[9] However, it may be accompanied with inadequate oxygen delivery. Thereafter, it is necessary to develop new oxygen administration strategy to improve resuscitation from HS.

In our previous study,[10] we proposed and evaluated gradually increased oxygen administration (GIOA, gradual acclimatization from hypoxemia to hyperoxia), which was found to ameliorate blood pressure, acid–base status, and oxygenation to an extent comparable to hyperoxic resuscitation, and mitigate oxidative stress to an extent comparable to hypoxemic resuscitation.

However, the effects of GIOA on survival remain unclear; and details of GIOA, such as whether hypoxemia at onset of resuscitation is imperative and the optimal timing of hypoxemic status are to be identified. We hypothesized that GIOA

Copyright © 2016 by the Society for Experimental Biology and Medicine

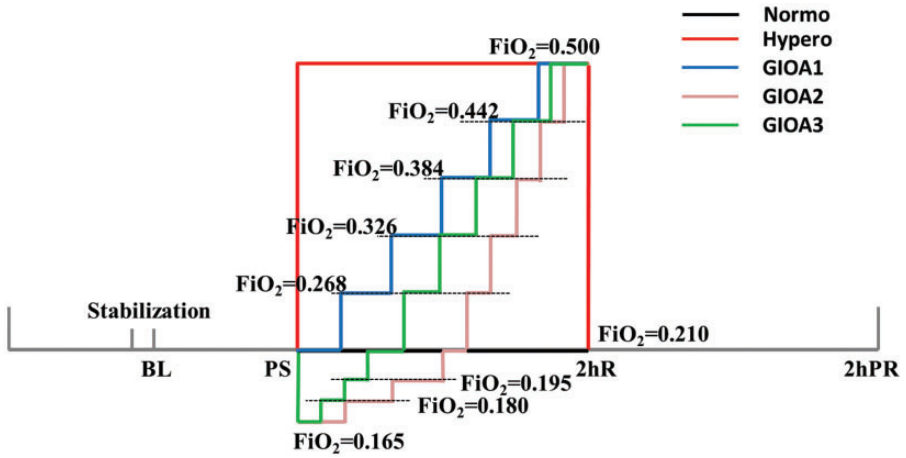

Figure 1 Experimental protocol. FiO_2, fraction of inspiration O_2. BL, baseline. PS, post-hemorrhagic shock. R, after the initiation of resuscitation. PR, post-resuscitation. At the end of hemorrhagic shock, rats were randomly assigned to one of the five groups: the normoxic group (Normo) in which the fraction of inspiration O_2 (FiO_2) was 0.210; the hyperoxic group (Hypero) in which the FiO_2 was 0.500; the GIOA group 1 (GIOA1), in which the FiO_2 increased from 0.210 to 0.500 by 0.058 per 20 min; the GIOA group 2 (GIOA2), in which the FiO_2 increased from 0.165 to 0.210 by 0.015 per 20 min, and from 0.210 to 0.500 by 0.058 per 10 min; and the GIOA group 3 (GIOA3), in which the FiO_2 increased from 0.165 to 0.210 by 0.015 per 10 min, and from 0.210 to 0.500 by 0.058 per 15 min. (A color version of this figure is available in the online journal.)

would promote survival, and hypoxemia at the early moments of resuscitation is critical to the benefits of GIOA in a rat HS model.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences, and conforms to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Eight-week-old male Wistar (300–340 g) rats were purchased from Vital River (Beijing, China). They fasted overnight but were allowed water *ad libitum* before experiments.

HS model

The HS model was performed as previously described.[10] Briefly, anesthesia was induced with sodium pentobarbital (50 mg/kg intraperitoneally), and was maintained every 90 min (20 mg/kg). The left femoral artery and vein were cannulated for blood withdrawal and drug infusions, the right femoral artery was cannulated for mean arterial pressure (MAP) monitoring, and the superior vena cava was cannulated for central venous blood collection. To prevent blood coagulation, 400 U/kg of heparin was administrated before bleeding, and 100 U/kg was administrated after resuscitation. Rats were bled to an MAP of 38 mmHg at the rate of 0.4 mL/min using a syringe pump (LZS – AJ10, Softron, Beijing, China), and this pressure was thereafter maintained at 35–40 mmHg for 60 min by reinfusion of the shed blood or further withdrawal at the rate of 0.3 mL/min.

Experimental groups

At the end of the HS, the rats were randomly assigned to one of the five groups ($n = 9$): the normoxic group (Normo) in which the fraction of inspiration O_2 (FiO_2) was 0.210; the hyperoxic group (Hypero) in which the FiO_2 was 0.500; the GIOA group 1 (GIOA1), in which the FiO_2 increased from 0.210 to 0.500 by 0.058 per 20 min; the GIOA group 2 (GIOA2), in which the FiO_2 increased from 0.165 to 0.210 by 0.015 per 20 min, and from 0.210 to 0.500 by 0.058 per 10 min; and the GIOA group 3 (GIOA3), in which the FiO_2 increased from 0.165 to 0.210 by 0.015 per 10 min, and from 0.210 to 0.500 by 0.058 per 15 min (Figure 1). The way FiO_2 changed was described in detail in our previous study.[10]

Meanwhile, the rats were resuscitated with three times the shed blood volume as Lactated Ringer's solution over a 2-h period. Experiments were continued 2 h after resuscitation for observation. At 2 h post-resuscitation (PR), the rats were sewed up, and moved to a regular cage. Survival was observed up to 96 h PR.

Measurement of hemodynamics and blood gases

Hemodynamics was continuously monitored via a multiple-channel recorder (MP150 Research System, Biopac System Inc., Montreal, Canada) throughout the experiment. At baseline (BL), post-hemorrhagic shock (PS), 1 h after the initiation of resuscitation (R), 2 h R, 1 h PR, and 2 h PR, 0.1 mL arterial blood and 0.1 mL central venous blood were drawn for the test of blood gases, separately. Blood gases were analyzed using a blood gas analyzer (ABL90, Radiometer, Copenhagen, Denmark) for central venous oxygen saturation ($ScvO_2$).

Test for plasma alanine transaminase and aspartate transaminase

To test plasma alanine transaminase (ALT), aspartate transaminase (AST), and oxidative stress, additional 2.3 mL arterial blood were drawn at 2 h PR, and then centrifuged at 1500*g* 4°C for 15 min. The drawn blood was replaced by a 1:1 volume of shed blood at all time points. The extracted plasma at 2 h PR were analyzed by measuring plasma ALT and AST using a biochemistry automatic analyzer (7180 Clinical Analyzer, Hitachi, Tokyo, Japan).

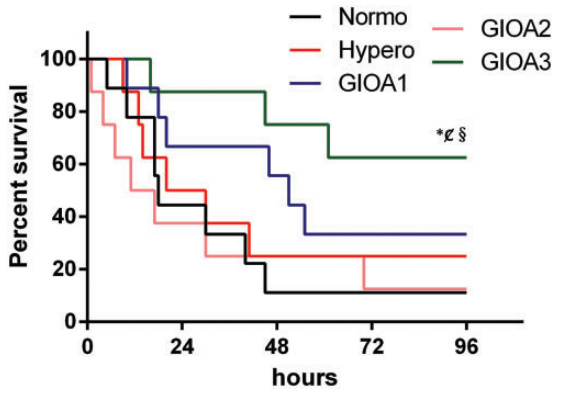

Figure 2 Effects on survival. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. *$P < 0.05$ Normo vs. GIOA3; ¢ $P < 0.05$ Hypero vs. GIOA3; § $P < 0.05$ GIOA2 vs. GIOA3. (A color version of this figure is available in the online journal.)

Test of oxidative stress

Plasma samples were tested with 3-nitrotyrosine (3-NT) assay kit (Z062SC, Elixir Canada Medicine Company Ltd, Vancouver, Canada) according to the manufacturer's instructions. SpectraMax M5 spectrophotometer (Molecular Devices, Sunnyvale, USA) was used.

Statistical analyses

All variables are presented as the mean ± SD. SAS 9.2 (SAS Institute Inc., Cary, NC) was used for statistical analysis. The investigators were blinded to the experimental group assignment: the people who adjusted the FiO_2, the people who recorded the data and the people who analyzed the data were all different. Since survival was the primary outcome variable, statistical power analysis was used to guide sample size estimation based on the survival ($\alpha = 0.05$, power = 80%). For $ScvO_2$ and MAP, two-way ANOVA repeated measures was used to compare the differences between groups and their interaction at various time points. For plasma 3-NT levels and organ function, ANOVA with Student–Newman–Keuls *post hoc* testing was used. Kaplan–Meier analysis was used to estimate survival. Differences were considered significant when $P < 0.05$.

Results

Effects on survival

Five rats (5/9) in the GIOA3 survived up to 96 h, compared to 1/9, 2/9, 3/9, and 1/9 in the Normo, Hypero, GIOA1, and GIOA2, respectively (Figure 2). The mean survival time of the GIOA3 (53.3 ± 6.5 h) was significantly longer than that of the Normo (25.3 ± 4.9 h), Hypero (24.5 ± 4.9 h), and GIOA2 (25.7 ± 10.2 h). The mean survival time of the GIOA1 was 40.1 ± 6.5 h.

Effects on ALT and AST

At 2 h PR, ALT concentration was significantly lower in the Normo (81 ± 24 U/L), GIOA1 (87 ± 38 U/L), and GIOA3 (75 ± 16 U/L) compared to the Hypero (116 ± 42 U/L) and GIOA2 (154 ± 59 U/L) ($P < 0.05$) (Figure 3a). Plasma AST concentration did not differ significantly between the groups at 2 h PR (Figure 3b).

Effects on oxidative stress and oxygenation

Plasma 3-NT levels at 2 h PR (Figure 4) were significantly lower in the GIOA2 (89.6 ± 17.7 ng/mL) and GIOA3 (94.7 ± 27.0 ng/mL) compared to the Normo (133.6 ± 33.6 ng/mL) and Hypero (133.4 ± 18.1 ng/mL) ($P < 0.05$).

$ScvO_2$ decreased from BL to PS similarly in all groups, and recovered upon resuscitation (Figure 5). At 1 h R, $ScvO_2$ in the Hypero (46.4 ± 8.0%) was significantly higher than the Normo (35.9 ± 5.6%), GIOA2 (31.7 ± 7.0%), and GIOA3 (39.2 ± 7.0%) ($P < 0.05$, respectively). At 2 h R, $ScvO_2$ in the Hypero (47.7 ± 12.8%) and GIOA groups were significantly higher than the Normo (35.9 ± 5.6%) ($P < 0.05$, respectively). At 1 h PR, $ScvO_2$ in the Hypero (43.4 ± 10.2%), GIOA1 (47.2 ± 9.8%), and GIOA3 (46.0 ± 9.8%) were significantly higher than the GIOA2 (33.8 ± 7.2%) ($P < 0.05$, respectively). At 2 h PR, $ScvO_2$ in the GIOA3 (52.0 ± 10.3%) were significantly higher than the Normo (41.8 ± 9.4%) and GIOA2 (35.3 ± 9.3%) ($P < 0.05$, respectively); besides, $ScvO_2$ in the GIOA1 (49.9 ± 7.5%) were significantly higher than the GIOA2 ($P < 0.05$).

Effects on blood gases

As shown in Table 1, there was no significant difference in acid–base status between the groups at BL and PS. Arterial pH, BE, lactate, and HCO_3^- values deteriorate during hemorrhage and recovered after resuscitation. At 2 h R, pH in the Normo, Hypero, GIOA1, and GIOA3 were significantly higher than the GIOA2. And at 2 h PR, BE in the Normo, Hypero, GIOA1, and GIOA3 were significantly higher than the GIOA2. At 1 h R, arterial lactate in the Hypero, GIOA1, and GIOA3 were significantly lower than the GIOA2.

In Table 2, arterial hemoglobin concentrations were comparable between groups at all timepoints. At 1 h R, PaO_2 in the Hypero were significantly higher than the Normo, GIOA1, GIOA2, and GIOA3. PaO_2 in the GIOA1 and GIOA3 were significantly higher than the Normo and GIOA2; in addition, PaO_2 in the GIOA1 were significantly higher than the GIOA3. At 2 h R, the PaO_2 of the Hypero and GIOA groups were significantly higher than those of the Normo. No significant differences in PaO_2 were observed at 1 h PR and 2 h PR.

Effects on hemodynamics

During HS, MAP decreased significantly to 35–40 mmHg in all of the rats (Figure 6a). The Hypero showed a faster increase of MAP compared to the Normo and GIOA groups until 1.5 h R. MAP in the GIOA3 were significantly lower compared to the Hypero at 0.5 h R, 1 h R, and 1.5 h R, respectively ($P < 0.05$, respectively). At 2 h R, MAP in the GIOA2 (81.1 ± 11.5 mmHg) was significantly lower than the Hypero (92.9 ± 7.4 mmHg), the GIOA1 (89.3 ± 5.8 mmHg), and the GIOA3 (90.9 ± 7.7 mmHg), respectively ($P < 0.05$, respectively); and MAP in the GIOA2 was significantly higher than the Normo (70.5 ± 3.5 mmHg) ($P < 0.05$).

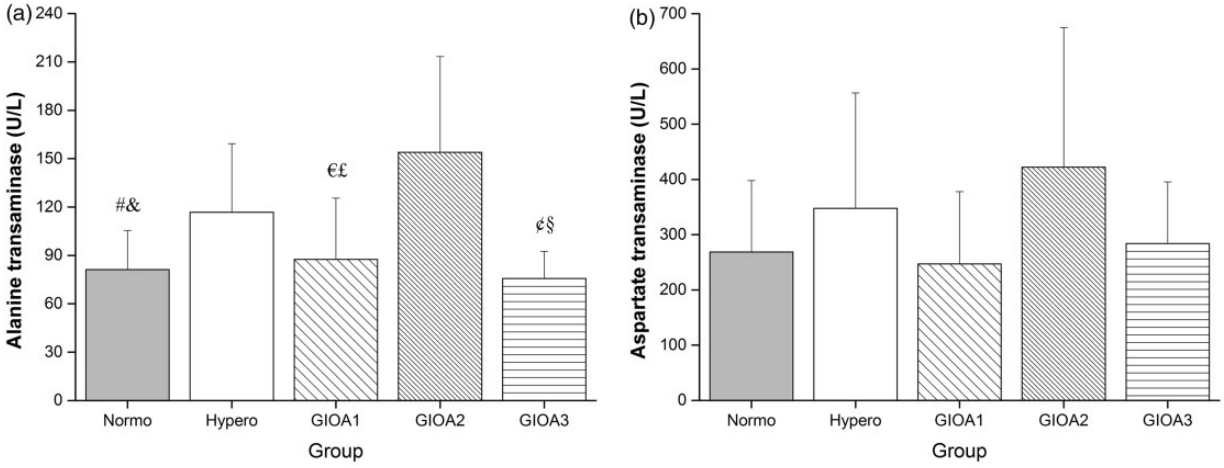

Figure 3 Effects on (a) plasma alanine transaminase (ALT) at 2 h post-resuscitation (PR); (b) plasma aspartate transaminase (AST) at 2 h PR. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. #$P < 0.05$ Normo vs. Hypero; &$P < 0.05$ Normo vs. GIOA2; €$P < 0.05$ Hypero vs. GIOA1; £$P < 0.05$ GIOA1 vs. GIOA2; ¢$P < 0.05$ Hypero vs. GIOA3; §$P < 0.05$ GIOA2 vs. GIOA3

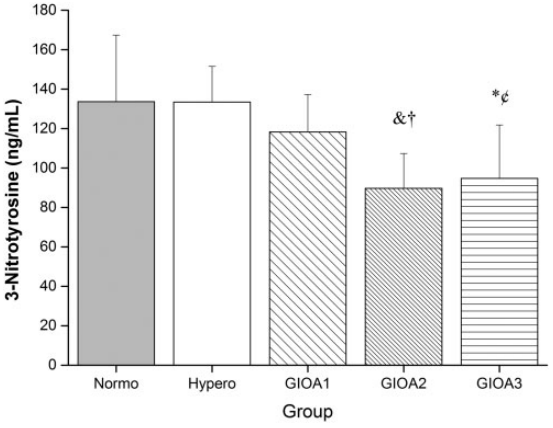

Figure 4 Effects on plasma 3-nitrotyrosine at 2 h post-resuscitation. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. &$P < 0.05$ Normo vs. GIOA2; †$P < 0.05$ Hypero vs. GIOA2; *$P < 0.05$ Normo vs. GIOA3; ¢ $P < 0.05$ Hypero vs. GIOA3

Besides, at 2 h PR, MAP were significantly higher ($P < 0.05$) in the Hypero (77.6 ± 7.1 mmHg), GIOA1 (77.3 ± 7.9 mmHg) and GIOA3 (81.0 ± 11.7 mmHg) compared to the Normo (66.9 ± 6.9 mmHg). And MAP in the GIOA3 was significantly higher ($P < 0.05$) than the GIOA2 (68.8 ± 15.6 mmHg) at 2 h PR. No significant differences between the Hypero and GIOA3 were observed after 1.5 h R.

HR showed an initial fall during hemorrhage, and increased to the baseline level by the end of shock (Figure 6b). There was no significant difference until 1 h R. No significant difference was observed at 2 h R. At 2 h PR, HR in the Hypero (491.8 ± 21.8 BPM), GIOA1 (509.8 ± 23.9 BPM), and GIOA3 (499.4 ± 20.4 BPM) was significantly higher than the Normo (471.4 ± 17.7 BPM), respectively ($P < 0.05$, respectively). In addition, HR in the Hypero, GIOA1, and GIOA3 was significantly higher than the GIOA2 (464.3 ± 15.2 BPM) at 2 h PR, respectively ($P < 0.05$, respectively).

Rate–pressure product (Figure 6c) was higher in the Hypero and GIOA1 compared to the Normo after 0.5 h R. After 2 h R, rate–pressure product was higher in the Hypero and GIOA3 compared to the GIOA2, and higher in the GIOA3 compared to the Normo.

Discussion

The major findings of the current study are that the GIOA3 showed improved hemodynamics and oxygenation compared to the Normo and GIOA2, meliorative oxidative stress compared to the Hypero, and reduced plasma ALT compared to the Hypero and GIOA2. The integrated effects of these factors may result in the improved survival of GIOA3 compared to the Normo, Hypero, and GIOA2.

In the present study, we did not compare hypoxemic resuscitation. This is because HS is characterized by impaired systemic/tissue oxygenation, and a high FiO_2 is imperative to supplement the oxygen deficiency. This was reflected in our previous study in that hypoxemic resuscitation did not show better results compared to the GIOA. Furthermore, hypoxemic resuscitation showed no difference in mortality compared to normoxemic resuscitation in Douzinas EE's study.[11] In addition, the current study infused LR during resuscitation rather than shed blood plus LR, as was done in our previous study; this is because blood is not available immediately in most cases.[12,13]

Concerns about whether hypoxemia at onset of resuscitation is imperative and the optimal timing of hypoxemic status exist.

GIOA1 increased FiO_2 from 0.210 to 0.500, and GIOA3 increased FiO_2 from 0.165 to 0.500. All parameters did not differ significantly between GIOA1 and GIOA3. However, at 2 h PR, MAP were comparable between GIOA1 and GIOA2, while GIOA3 was significantly higher than GIOA2; $ScvO_2$ were comparable between GIOA1 and Normo, while GIOA3 was significantly higher than the Normo; and no significant difference was observed between GIOA1, Normo, and Hypero in 3-NT, while

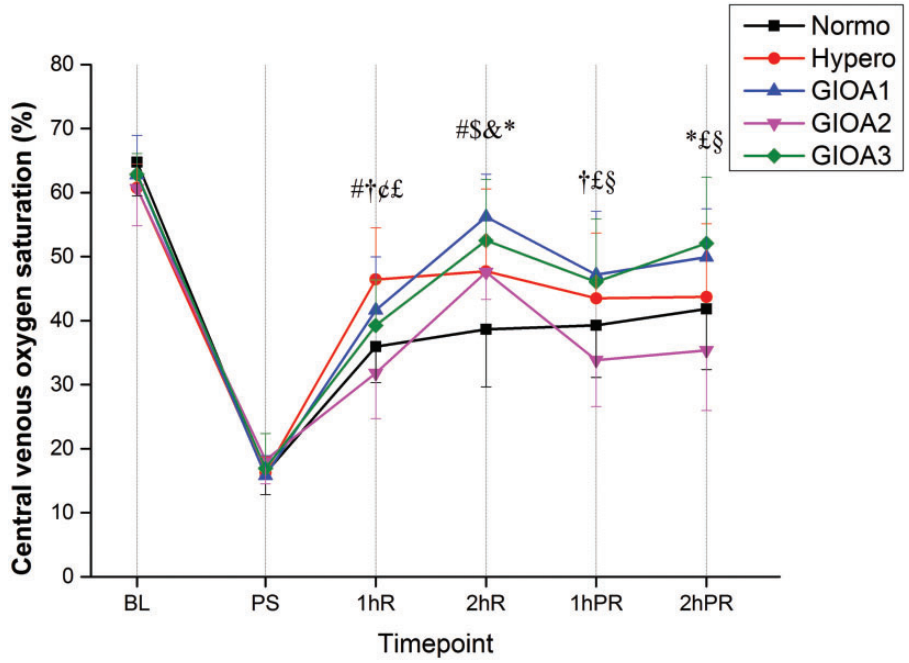

Figure 5 Effects on central venous oxygen saturation. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. BL, baseline. PS, post-hemorrhagic shock. R, after the initiation of resuscitation. PR, post-resuscitation. #$P < 0.05$ Normo vs. Hypero; \$$P < 0.05$ Normo vs. GIOA1; &$P < 0.05$ Normo vs. GIOA2; *$P < 0.05$ Normo vs. GIOA3; †$P < 0.05$ Hypero vs. GIOA2; ¢ $P < 0.05$ Hypero vs. GIOA3; £$P < 0.05$ GIOA1 vs. GIOA2; §$P < 0.05$ GIOA2 vs. GIOA3. (A color version of this figure is available in the online journal.)

Table 1 Acid–base status at different time points during the experiment

		Normo	Hypero	GIOA1	GIOA2	GIOA3
pH	BL	7.41 ± 0.01	7.41 ± 0.02	7.41 ± 0.01	7.41 ± 0.01	7.42 ± 0.02
	PS	7.33 ± 0.04	7.35 ± 0.02	7.34 ± 0.02	7.32 ± 0.03	7.34 ± 0.06
	60'R	7.36 ± 0.06	7.39 ± 0.05†	7.36 ± 0.05	7.31 ± 0.01	7.38 ± 0.07
	120'R	7.47 ± 0.06$^{\&}$	7.46 ± 0.02†	7.45 ± 0.03$^{£}$	7.38 ± 0.01	7.45 ± 0.02§
	60'PR	7.46 ± 0.04	7.47 ± 0.02	7.46 ± 0.03	7.44 ± 0.04	7.46 ± 0.02
	120'PR	7.43 ± 0.10	7.47 ± 0.02	7.47 ± 0.01	7.42 ± 0.03	7.46 ± 0.03
BE (mmol/L)	BL	−1.7 ± 2.7	−2.5 ± 2.1	−2.7 ± 1.6	−2.5 ± 1.4	−0.5 ± 1.8
	PS	−12.3 ± 2.8	−11.0 ± 2.0	−11.1 ± 1.8	−11.5 ± 2.4	−11.5 ± 3.6
	60'R	−8.2 ± 4.6	−5.8 ± 2.4†	−5.3 ± 3.0$^{£}$	−10.0 ± 5.7	−7.0 ± 4.5
	120'R	−1.5 ± 4.65$^{\&}$	−0.01 ± 2.1†	−0.2 ± 1.7$^{£}$	−4.9 ± 4.9	−2.0 ± 2.4
	60'PR	−2.7 ± 4.0	−1.2 ± 2.4†	−2.0 ± 1.3$^{£}$	−4.5 ± 3.5	−2.2 ± 2.6§
	120'PR	−2.3 ± 4.3$^{\&}$	−2.2 ± 2.3†	−1.3 ± 2.4$^{£}$	−5.0 ± 3.1	−2.0 ± 2.7§
Lac (mmol/L)	BL	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 1.3	0.5 ± 0.1
	PS	7.2 ± 1.7	7.1 ± 1.9	6.5 ± 1.5	7.9 ± 1.1	8.2 ± 2.8
	60'R	6.2 ± 3.2	3.0 ± 1.5†	5.0 ± 2.3	7.7 ± 3.0	5.1 ± 2.8
	120'R	2.5 ± 1.4	1.4 ± 0.8†	1.1 ± 0.5$^{£}$	3.2 ± 3.2	1.7 ± 0.9
	60'PR	2.1 ± 1.4	1.1 ± 0.9†	1.3 ± 1.2$^{£}$	2.3 ± 1.6	1.4 ± 0.7§
	120'PR	2.2 ± 1.7	1.3 ± 1.0	1.2 ± 1.2	2.2 ± 1.9	1.2 ± 0.9
HCO_3^-(mmol/L)	BL	21.8 ± 3.5	21.1 ± 2.8	21.4 ± 1.7	21.8 ± 1.4	23.8 ± 2.1
	PS	12.2 ± 2.7	13.3 ± 1.9	13.4 ± 1.9	13.1 ± 2.0	12.9 ± 3.0
	60'R	16.4 ± 4.1	18.5 ± 2.0	19.5 ± 2.8	15.2 ± 4.6	14.7 ± 8.5
	120'R	22.2 ± 4.3	23.4 ± 2.1	23.8 ± 2.0	19.5 ± 4.4	19.1 ± 8.2
	60'PR	21.0 ± 3.8	22.5 ± 2.3	21.6 ± 1.2	19.3 ± 3.3	18.8 ± 8.9
	120'PR	21.8 ± 4.2	21.4 ± 2.3	22.3 ± 2.4	19.0 ± 2.9	19.2 ± 9.1

Values are presented as the mean ± SD. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. BL, baseline. PS, post-hemorrhagic shock. R, after the initiation of resuscitation. PR, post-resuscitation. BE, base excess; Lac, lactate.
$^{\&}P < 0.05$ Normo vs. GIOA2; $^{\dagger}P < 0.05$ Hypero vs. GIOA2; $^{£}P < 0.05$ GIOA1 vs. GIOA2; $^{\S}P < 0.05$ GIOA2 vs. GIOA3.

Table 2 Gas exchange at different time points during the experiment

		Normo	Hypero	GIOA1	GIOA2	GIOA3
Hb (g/dL)	BL	12.5 ± 1.4	11.9 ± 1.3	12.2 ± 0.9	12.5 ± 1.0	12.7 ± 0.5
	PS	10.7 ± 1.3	11.8 ± 0.9	11.3 ± 1.4	11.8 ± 1.0	11.4 ± 0.9
	60'R	7.3 ± 1.2	7.1 ± 0.6	8.0 ± 0.5	7.7 ± 1.2	7.7 ± 0.9
	120'R	6.9 ± 1.3	7.1 ± 0.8	6.8 ± 0.5	6.4 ± 0.7	6.5 ± 0.8
	60'PR	6.7 ± 0.9	6.7 ± 0.4	6.7 ± 0.5	6.8 ± 1.0	7.2 ± 0.5
	120'PR	6.9 ± 1.1	6.6 ± 0.7	6.8 ± 0.4	6.7 ± 1.1	6.8 ± 0.7
PaO_2 (mmHg)	BL	85.1 ± 7.5	80.9 ± 4.8	82.5 ± 7.0	82.8 ± 7.1	80.1 ± 5.0
	PS	122.1 ± 5.5	119.3 ± 7.5	118.2 ± 7.5	118.3 ± 7.6	118.1 ± 8.7
	60'R	110.8 ± 12.2	208.4 ± 29.3$^{\#\in\dagger¢}$	162.3 ± 8.7$^{\$£¥}$	95.9 ± 17.7	137.0 ± 15.8$^{*\$}$
	120'R	102.2 ± 9.5	206.0 ± 23.8$^{\#}$	205.6 ± 19.9$^{\$}$	206.8 ± 17.7$^{\&}$	200.4 ± 26.2*
	60'PR	102.9 ± 10.3	96.3 ± 11.6	100.1 ± 9.1	103.9 ± 9.3	96.4 ± 9.9
	120'PR	106.4 ± 10.0	95.5 ± 15.4	94.5 ± 6.9	102.5 ± 11.9	96.3 ± 4.5
PvO_2 (mmHg)	BL	51.1 ± 5.8	48.6 ± 3.1	50.4 ± 4.4	48.4 ± 4.3	49.2 ± 2.8
	PS	28.2 ± 6.8	24.7 ± 2.6	26.6 ± 6.8	26.5 ± 4.0	22.2 ± 5.3*
	60'R	36.8 ± 4.0	40.2 ± 4.1	39.3 ± 3.9	38.0 ± 9.0	36.6 ± 3.0
	120'R	34.1 ± 4.9	37.4 ± 8.3	42.7 ± 3.5$^{\$}$	42.5 ± 6.0$^{\&}$	39.6 ± 6.5
	60'PR	32.0 ± 3.5	33.2 ± 5.4	36.8 ± 5.9	31.4 ± 4.4$^{£}$	35.9 ± 6.7
	120'PR	35.3 ± 4.4	33.9 ± 6.0	37.5 ± 4.4	31.7 ± 4.2	38.3 ± 7.0§
$PaCO_2$ (mmHg)	BL	33.7 ± 5.4	33.3 ± 4.5	32.9 ± 2.4	34.4 ± 1.6	36.7 ± 3.9
	PS	22.8 ± 4.8	24.0 ± 2.7	24.7 ± 3.9	24.8 ± 2.9	23.2 ± 3.9
	60'R	28.3 ± 4.5	30.2 ± 3.5	34.1 ± 5.0$^{£¥}$	28.7 ± 4.5	28.8 ± 3.6
	120'R	29.9 ± 5.0	32.9 ± 4.4	34.5 ± 5.3$^{\$}$	30.8 ± 9.1$^{\&}$	30.7 ± 3.5
	60'PR	29.2 ± 4.0	30.6 ± 2.3	29.8 ± 2.3	28.0 ± 3.9	29.8 ± 3.0
	120'PR	29.1 ± 3.5	29.2 ± 3.0	30.1 ± 2.9	28.8 ± 3.6	29.9 ± 2.3
$PvCO_2$ (mmHg)	BL	41.4 ± 5.7	42.6 ± 5.6	44.7 ± 3.3	43.4 ± 4.1	41.9 ± 3.1
	PS	40.3 ± 6.7	43.9 ± 6.4	46.3 ± 4.3$^{\$}$	42.7 ± 4.5	41.5 ± 5.4
	60'R	38.0 ± 4.3	43.0 ± 4.7$^{\#¢}$	45.1 ± 3.3$^{\$£¥}$	39.7 ± 3.6	37.5 ± 4.0
	120'R	35.9 ± 3.8	40.9 ± 2.8$^{\#}$	40.5 ± 2.7$^{\$}$	39.4 ± 5.6	37.4 ± 2.4
	60'PR	37.6 ± 4.0	36.4 ± 2.7	37.1 ± 3.4	37.0 ± 4.7	35.3 ± 2.8
	120'PR	38.5 ± 2.8	35.8 ± 3.4	38.4 ± 1.5	39.3 ± 3.5	35.1 ± 2.2$^{*¥\S}$

Values are presented as the mean ± SD. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. BL, baseline. PS, post-hemorrhagic shock. R, after the initiation of resuscitation. PR, post-resuscitation. Hb, hemoglobin; PaO_2, arterial O_2 partial pressure; PvO_2, central venous O_2 partial pressure; $PaCO_2$, arterial CO_2 partial pressure; $PvCO_2$, central venous CO_2 partial pressure.

$^{\#}P < 0.05$ Normo vs. Hypero; $^{\$}P < 0.05$ Normo vs. GIOA1; $^{\&}P < 0.05$ Normo vs. GIOA2; $^{*}P < 0.05$ Normo vs. GIOA3; $^{\in}P < 0.05$ Hypero vs. GIOA1; $^{\dagger}P < 0.05$ Hypero vs. GIOA2; $^{¢}P < 0.05$ Hypero vs. GIOA3; $^{£}P < 0.05$ GIOA1 vs. GIOA2; $^{¥}P < 0.05$ GIOA1 vs. GIOA3; $^{\S}P < 0.05$ GIOA2 vs. GIOA3.

GIOA3 was significantly lower than the Normo and Hypero. All of these may lead to the comparable survival of GIOA1, Normo, Hypero, and GIOA2, while the better survival of GIOA3 compared to the Normo, Hypero, and GIOA2. To be noted, GIOA1 emphasizes the gradual process, while GIOA3 not only emphasizes the gradual process, but also focuses on the hypoxemic status at the onset of reperfusion. We may thereafter conclude that the hypoxemic status at the onset of reperfusion may be imperative in the benefits of GIOA. And further research are required to determine the optimal initial FiO_2.

GIOA2 and GIOA3 both increased FiO_2 from 0.165 to 0.500; however, the hypoxemic status last 1 h in GIOA2, compared to 0.5 h in GIOA3. The liver is among the most frequently affected organs after HS.[14] In our previous study, GIOA increased liver oxygenation and decreased liver injury simultaneously. In the current study, GIOA3 showed reduced plasma ALT compared to GIOA2. Besides, GIOA3 showed better hemodynamics, acid-base balance, and survival compared to GIOA2. From these results, we may conclude that long-time hypoxemic status during resuscitation is harmful, and early moments of GIOA are critical to the benefits.

HS is associated with decreased blood flow, impaired oxygenation, increased oxidative stress, and organ dysfunction. The current biomedical strategy has developed significantly to target the unique proximal causes of the pathologies to alleviate the signs of HS.[15] For example, crystalloid and colloid are infused to supplement the blood volume during HS, and $NaHCO_3$ is used to treat metabolic acidosis.[16] However, these strategies treat each of the pathologies separately,[15] and a resuscitation strategy that provides multiple therapeutic effects may be preferred. Combining the results of the current study and our

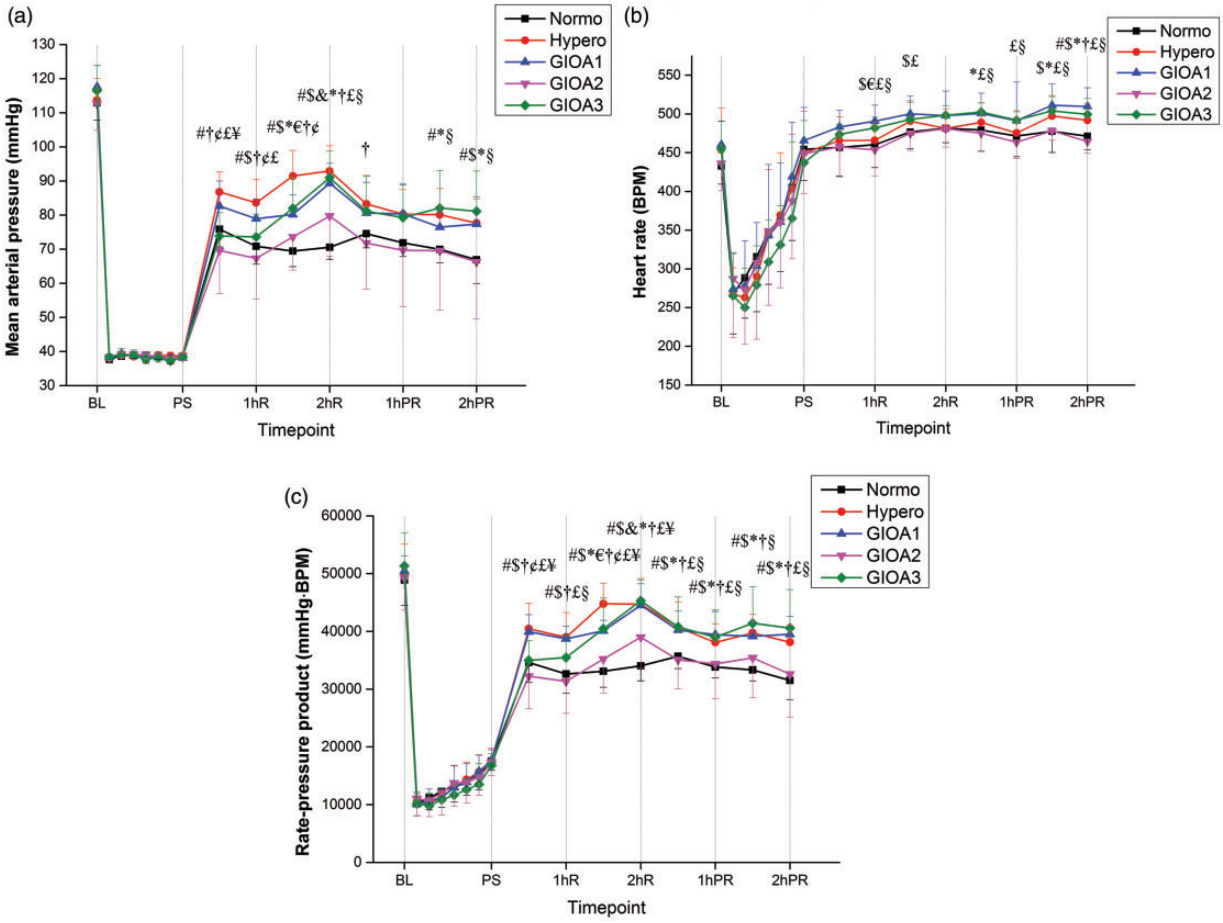

Figure 6 Mean arterial pressure (a), heart rate (b), and rate-pressure product (c) at different time points during the experiment. Normo, the normoxic group; Hypero, the hyperoxic group; GIOA1, the GIOA group 1; GIOA2, the GIOA group 2; GIOA3, the GIOA group 3. BL, baseline. PS, post-hemorrhagic shock. R, after the initiation of resuscitation. PR, post-resuscitation. #$P < 0.05$ Normo vs. Hypero; \$$P < 0.05$ Normo vs. GIOA1; &$P < 0.05$ Normo vs. GIOA2; *$P < 0.05$ Normo vs. GIOA3; € $P < 0.05$ Hypero vs. GIOA1; †$P < 0.05$ Hypero vs. GIOA2; ¢ $P < 0.05$ Hypero vs. GIOA3; £$P < 0.05$ GIOA1 vs. GIOA2; ¥$P < 0.05$ GIOA1 vs. GIOA3; §$P < 0.05$ GIOA2 vs. GIOA3. (A color version of this figure is available in the online journal.)

previous study, we can conclude that GIOA may simultaneously restore hemodynamics, ameliorate acid–base status, improve systemic/tissue oxygenation, mitigate liver oxidative stress, reduce plasma ALT, and prolong survival time.

Just like the story of oxygen in HS, asphyxiated neonates were resuscitated with oxygen since 1780.[17] However, many researchers and clinicians questioned the use of oxygen for neonatal resuscitation. Studies showed that infants resuscitated with air showed a significant benefit compared to 100% oxygen,[18] and asphyxiated neonates resuscitated with oxygen resulted in significantly higher oxidative stress compared to air.[6,7] Thereafter, the guideline for the use of oxygen during neonatal resuscitation has been revised and recommended the use of room air since 2010. What's more, efforts have been made to investigate the effects of resuscitation with lower O_2 concentrations.[19,20] Meanwhile, concerns have been raised that severely asphyxiated neonates resuscitated with room air may be accompanied with inadequate oxygen delivery.[21] Thus, we believe that improving oxygenation and mitigating oxidative stress simultaneously is also a critical issue in neonatal resuscitation, and GIOA (maybe from normoxia to hyperoxia) is likely to show good results. We will study the effects of GIOA in neonatal resuscitation in our further research.

The current study has some limitations. Wistar rats were used in the study, the effects of GIOA on larger animals remain unclear. Besides, metabolomics could help identify important pathway activated by different oxygen concentration, and it is useful for monitoring the experiment. Last but not least, parameters are necessary to indicate the optimal initial oxygen concentration of GIOA and the rate FiO_2 increased. We will work on these limitations in our further research.

In conclusion, the current study showed that (1) GIOA could significantly prolong survival time compared to normoxemic resuscitation and hyperoxic resuscitation; (2) early moments of GIOA are critical to the benefits; and (3) hypoxemia at onset of resuscitation may be imperative, more works are needed to determine the optimal initial oxygen concentration of GIOA.

Author contributions: XL conceived and designed the study, performed the experiments, analyzed the data, and drafted the manuscript. GC, GY, BW, and ML assisted in the

preparation of the experimental animal models and supervised the data collection. JZ and YW acquired and analyzed the data and revised the manuscript. YY, LZ, and HZ conceived the study, revised the manuscript critically for important intellectual content, and approved the final version to be published.

ACKNOWLEDGMENTS

The authors thank Weiwei Liu, PhD (Consulting Center of Biomedical Statistics, Academy of Military Medical Sciences, Beijing, China) for his advices regarding the data analysis. This work was completed with financial support from the National High Technology Research and Development Program of China (2012AA021902) and National Natural Science Foundation of China (81300751).

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Angele MK, Schneider CP, Chaudry IH. Bench-to-bedside review: latest results in hemorrhagic shock. *Crit Care (London, England)* 2008;**12**:218
2. Dutton RP. Current concepts in hemorrhagic shock. *Anesthesiol Clin* 2007;**25**:23–34. (viii)
3. Leonov Y, Safar P, Sterz F, Stezoski SW. Extending the golden hour of hemorrhagic shock tolerance with oxygen plus hypothermia in awake rats. An exploratory study. *Resuscitation* 2002;**52**:193–202
4. Sjoberg F, Singer M. The medical use of oxygen: a time for critical reappraisal. *J Intern Med* 2013;**274**:505–28
5. Branson RD, Johannigman JA. Pre-hospital oxygen therapy. *Respir Care* 2013;**58**:86–97
6. Tataranno ML, Oei JL, Perrone S, Wright IM, Smyth JP, Lui K, Tarnow-Mordi WO, Longini M, Proietti F, Negro S, Saugstad OD, Buonocore G. Resuscitating preterm infants with 100% oxygen is associated with higher oxidative stress than room air. *Acta Paediatr (Oslo, Norway: 1992)* 2015;**104**:759–65
7. Walson KH, Tang M, Glumac A, Alexander H, Manole MD, Ma L, Hsia CJ, Clark RS, Kochanek PM, Kagan VE, Bayr H. Normoxic versus hyperoxic resuscitation in pediatric asphyxial cardiac arrest: effects on oxidative stress. *Crit Care Med* 2011;**39**:335–43
8. Dyson A, Stidwill R, Taylor V, Singer M. The impact of inspired oxygen concentration on tissue oxygenation during progressive haemorrhage. *Intensive Care Med* 2009;**35**:1783–91
9. Douzinas EE, Livaditi O, Xiarchos AG, Giamarellos-Bourboulis EJ, Villiotou V, Liappas IA, Evangelou E, Rapidis AD, Roussos C. The effect of hypoxemic resuscitation of hemorrhagic shock on hemodynamic stabilization and inflammatory response: a pilot study in a rat experimental model. *J Trauma* 2006;**61**:918–23
10. Luo X, Yin Y, You G, Chen G, Wang Y, Zhao J, Wang B, Zhao L, Zhou H. Gradually increased oxygen administration improved oxygenation and mitigated oxidative stress after resuscitation from severe hemorrhagic shock. *Anesthesiology* 2015;**123**:1122–32
11. Douzinas EE, Livaditi O, Andrianakis I, Prigouris P, Paneris P, Villiotou V, Betrosian AP. The effect of hypoxemic resuscitation from hemorrhagic shock on blood pressure restoration and on oxidative and inflammatory responses. *Intensive Care Med* 2008;**34**:1133–41
12. Developing countries face safe blood shortage. *Bull World Health Organ* 2004;**82**:558
13. Seifried E, Mueller MM. The present and future of transfusion medicine. *Blood Transfus* 2011;**9**:371–6
14. Heckbert SR, Vedder NB, Hoffman W, Winn RK, Hudson LD, Jurkovich GJ, Copass MK, Harlan JM, Rice CL, Maier RV. Outcome after hemorrhagic shock in trauma patients. *J Trauma* 1998;**45**:545–9
15. Burkewitz K, Zhang Y, Mair WB. AMPK at the nexus of energetics and aging. *Cell Metab* 2014;**20**:10–25
16. Adeva-Andany MM, Fernandez-Fernandez C, Mourino-Bayolo D, Castro-Quintela E, Dominguez-Montero A. Sodium bicarbonate therapy in patients with metabolic acidosis. *Sci World J* 2014;**2014**:627673
17. Obladen M. History of neonatal resuscitation. Part 2: oxygen and other drugs. *Neonatology* 2009;**95**:91–6
18. Davis PG, Tan A, O'Donnell CP, Schulze A. Resuscitation of newborn infants with 100% oxygen or air: a systematic review and meta-analysis. *Lancet* 2004;**364**:1329–33
19. Cheung PY, Obaid L, Emara M, Brierley Y, Johnson ST, Chan GS, Jewell L, Korbutt G, Bigam DL. Cardio-renal recovery of hypoxic newborn pigs after 18%, 21% and 100% reoxygenation. *Intensive Care Med* 2008;**34**:1114–21
20. Jantzie LL, Cheung PY, Obaid L, Emara M, Johnson ST, Bigam DL, Todd KG. Persistent neurochemical changes in neonatal piglets after hypoxia-ischemia and resuscitation with 100%, 21% or 18% oxygen. *Resuscitation* 2008;**77**:111–20
21. Kattwinkel J. Evaluating resuscitation practices on the basis of evidence: the findings at first glance may seem illogical. *J Pediatr* 2003;**142**:221–2

(Received December 30, 2015, Accepted March 18, 2016)