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Fresh Blood and Aged Stored Blood Are Equally Efficacious in Immediately Reversing Anemia-induced Brain Oxygenation Deficits in Humans

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Background: **Erythrocytes are transfused to treat or prevent imminent inadequate tissue oxygenation. 2,3-diphosphoglycerate concentration decreases and oxygen affinity of hemoglobin increases (P50 decreases) with blood storage, leading some to propose that erythrocytes stored for 14 or more days do not release sufficient oxygen to make their transfusion efficacious. The authors tested the hypothesis that erythrocytes stored for 3 weeks are as effective in supplying oxygen to human tissues as are erythrocytes stored for less than 5 h.**

Methods: **Nine healthy volunteers donated 2 units of blood more than 3 weeks before they were tested with a standard, computerized neuropsychological test (digit–symbol substitution test [DSST]) on 2 days, 1 week apart, before and after acute isovolemic reduction of their hemoglobin concentration to 7.4 and 5.5 g/dl. Volunteers randomly received autologous erythrocytes stored for either less than 5 h ("fresh") or 3 weeks ("stored") to return their hemoglobin concentration to 7.5 g/dl (double blinded). Erythrocytes of the alternate storage duration were transfused on the second experimental day. The DSST was repeated after transfusion.**

Results: **Acute anemia slowed DSST performance equivalently in both groups. Transfusion of stored erythrocytes with decreased P50 reversed the altered DSST ($P < 0.001$) to a time that did not differ from that at 7.4 g/dl hemoglobin during production of acute anemia ($P = 0.88$). The erythrocyte transfusion–induced DSST improvement did not differ between groups ($P = 0.96$).**

Conclusion: **Erythrocytes stored for 3 weeks are as efficacious as are erythrocytes stored for 3.5 h in reversing the neurocognitive deficit of acute anemia. Requiring fresh rather than stored erythrocytes for augmentation of oxygen delivery does not seem warranted.**

This article is accompanied by an Editorial View. Please see: Spahn DR, Madjdpour C: Physiologic transfusion triggers: Do we have to use (our) brain? ANESTHESIOLOGY 2006; 104:905–6.

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ERYTHROCYTES are transfused to treat or prevent imminent inadequate oxygen delivery and tissue oxygenation, or to improve symptoms of acute anemia. However, there have been no prospective randomized clinical trials in humans to document the efficacy of transfusion of stored blood. Survival after massive hemorrhage and transfusion of volumes equivalent to several blood volumes would seem to indicate that stored blood delivers sufficient oxygen to tissues to permit individual organ and overall survival.

Erythrocyte concentrations of 2,3-diphosphoglycerate (2,3-DPG) decrease with duration of erythrocyte storage,[1–10] reaching depleted concentrations of 1 μmol/g hemoglobin or less at 21 days of storage.[2,3,5,6,8] 2,3-DPG is an important modulator of the affinity of hemoglobin for oxygen.[11–14] The oxygen affinity of hemoglobin varies inversely with 2,3-DPG concentration (decreasing 2,3-DPG concentration decreases P50, the partial pressure of oxygen [P_{O_2}] at pH 7.4 and partial pressure of carbon dioxide [P_{CO_2}] 40 mmHg, at which the oxyhemoglobin saturation [S_{O_2}] is 50%). When erythrocytic 2,3-DPG is depleted, P50 is 16–20 mmHg,[8,10] rather than the normal 26.7 mmHg.[15] Some have suggested that the increased oxygen affinity of hemoglobin of stored erythrocytes does not allow for release of sufficient amounts of oxygen to tissues,[16] thus making erythrocytes stored for more than 14 days lacking in immediate efficacy, until 2,3-DPG concentrations and P50 are restored, some hours after transfusion.[2,3,5,16,17]

We have previously demonstrated that fresh blood, stored in citrate-phosphate-dextrose-adenine (CPDA-1) for less than 4 h, effectively reverses cognitive deficits in humans made acutely anemic to hemoglobin concentrations of 5 and 6 g/dl.[18] Those data demonstrate that erythrocytes stored for a few hours release quantities of oxygen that do not differ substantially from erythrocytes that have not been removed from the circulation. In the experiment described here, we used our model in which cerebral function is oxygen-delivery dependent, to test the null hypothesis that erythrocytes stored for at least 3 weeks are as effective as erythrocytes stored for 4 h in supplying oxygen to human tissues.

Materials and Methods

With approval of our institutional review board (University of California, San Francisco [UCSF]) and informed

consent, we studied nine paid volunteers who were without cardiovascular, pulmonary, or hepatic disease; did not smoke; were not taking any medications; and weighed less than 80 kg. The weight requirement was imposed to avoid excessively long experimental days, with potentially increased effects of time, owing to the need to remove large quantities of blood to achieve the desired hemoglobin concentration. A minimum of 80% correct responses for the neurocognitive test (see Digit–Symbol Substitution Test section) was required for participation in the study. Volunteers were required to have a minimum hemoglobin concentration of 12 g/dl before the first donation and 9.5 g/dl on each study day (see this section below), and vision that was normal with or without corrective lenses.

Four hundred fifty milliliters (one "unit") of blood was collected from each volunteer into CPDA-1 collection bags (Baxter Healthcare Corp., Deerfield, IL) 4–5 weeks before the first study day, and a second unit of blood was collected 4–8 days later. Each volunteer was studied on 2 separate days, separated by 7 days. We produced acute severe isovolemic anemia identically on each day, as previously described.[18–20] A radial arterial and two peripheral venous cannulae were inserted in each subject using local anesthesia. After insertion of the cannulae, subjects rested for 30 min before measurement of variables. The digit–symbol substitution test (DSST) (see Digit–Symbol Substitution Test section) was performed with the subject in a semisitting position before removal of any blood, and after producing isovolemic anemia to blood hemoglobin concentrations of 7 and 5 g/dl by removal of 450 ml blood into CPDA-1 collections bags. Removal of each 450 ml blood required approximately 10–15 min. To maintain isovolemia and compensate for the extravascular distribution of albumin,[19,21] simultaneous with blood withdrawal, 5% human serum albumin (American Red Cross/Baxter, Glendale CA) and the subject's own platelet-rich plasma (after separation from the erythrocytes of the removed blood) were infused intravenously in volumes of 19% (11–28%) (mean and 95% confidence interval) on the day "fresh" erythrocytes were transfused (see this section below) and 19% (10–28%) on the day "stored" erythrocytes were transfused, greater than that of the removed blood. A 5-min equilibration period was allowed after blood withdrawal, at each hemoglobin concentration, before the DSST was performed. Volunteers were monitored with continuous electrocardiography, blood pressure monitoring, and pulse oximetry.

After completion of the tests at hemoglobin concentration of 5 g/dl, a sufficient quantity of each volunteer's erythrocytes (not leukoreduced) was transfused, using a blood warmer, to return blood hemoglobin concentration to 7 g/dl, and the DSST was repeated. Fresh and stored erythrocytes were transfused as packed cells, prepared by the UCSF blood bank, using standard methods. Volunteers were randomly allocated (by a computer-generated list) to receive either erythrocytes withdrawn that day ("fresh," stored for less than 5 h) or autologous erythrocytes stored for at least 3 weeks ("stored") on the first experimental day. The erythrocytes of alternate storage time were transfused on the second experimental day. On both experimental days, the older of the two similar types of units of erythrocytes was always transfused first. The volunteers remained in the hospital overnight while all remaining erythrocytes that had been withdrawn that day were transfused. The volunteers and all study personnel, except the study coordinator, blood bank personnel preparing the erythrocytes for transfusion, and the physician transfusing the erythrocytes, were blinded to volunteer group assignment.

At each test period, arterial blood gases and pH (Radiometer ABL 505; Radiometer, Copenhagen, Denmark), oxyhemoglobin saturation (OSM3 Hemoximeter; Radiometer), and heart rate were measured, and the volunteer self-assessed his or her energy level, using a 10-cm visual analog scale. 2,3-DPG concentration was measured in a sample of each volunteer's blood at baseline. Blood was sampled from each unit of fresh and stored blood, immediately before transfusion, for measurement of P_{O_2}, P_{CO_2}, pH, S_{O_2}, and 2,3-DPG concentration. 2,3-DPG concentration was measured in duplicate in neutralized supernatants of deproteinated heparinized whole blood frozen at −80°C until analyzed using an enzymatic spectrophotometric method (Roche Diagnostics GmbH, Mannheim, Germany; catalog No. 148 334). The P50 of each sample of transfused blood and the volunteer's arterial blood were calculated according to Severinghaus' modification (John W. Severinghaus, M.D., Professor, Department of Anesthesia, UCSF) of his method[15] and included a correction for base-excess. This modification uses Ellis' mathematical solution[22] of Roughton and Severinghaus' modification[23] of the Hill equation for the relation between P_{O_2} and S_{O_2}, allowing for precise determination of S_{O_2} from a measured P_{O_2}. To accomplish this, we equilibrated each blood sample with a gas mixture to produce a blood sample for analysis that had an oxyhemoglobin saturation of 40–60% and a P_{CO_2} of 30–50 mmHg.

Digit–Symbol Substitution Test

The person administering the cognitive test was blinded to the group assignment of the volunteer. Speed of information processing for the DSST was assessed at each time point using the NES-2 computerized test (NES2, version 4.75; Neurobehavioral Systems Inc., Winchester, MA). These tests were presented with a computer and a 15-in monitor positioned approximately 65 cm from the subject. Subjects responded using a keyboard placed in their laps. Subjects were asked to respond as quickly as they could without making mistakes. The tests were administered once each on each day of

Table 1. Values before Hemodilution

Variable	Fresh	Stored	*P* Value
Hemoglobin, g/dl	11.7 (10.7–12.7)	11.8 (11.1–12.5)	0.55
P50, mmHg	27.7 (27.1–28.3)	27.4 (26.7–28.1)	0.18
DSST, ms	154 (142–167)	157 (142–173)	0.60
Energy	7.6 (5.8–9.3)	6.6 (5.0–8.2)	0.43
Heart rate, beats/min	65 (57–73)	67 (61–74)	0.39

Values are mean (95% confidence interval). For hemoglobin, P50 (hemoglobin affinity for oxygen; see text for complete definition), and digit–symbol substitution test (DSST), n = 8. For energy (self-assessed energy level) and heart rate, n = 9. *P* is probability of difference between fresh and stored. See text for additional statistical analyses.

testing, before insertion of intravascular cannulae, and twice before the first test day to familiarize subjects with the procedures and minimize postbaseline increments in performance caused by practice effects. This computerized test has been described previously.[18,20] Subjects were shown nine symbol–digit pairs at the top of the screen. A test set of the nine symbols was presented in the center of the screen in a scrambled order. Subjects were required to press the digits on the keyboard corresponding to the symbol in the test set. There were one practice set and three test sets of symbol–digit pairs.

Data Analysis and Statistics

The number of volunteers to be studied was determined *a priori* by a power analysis, using data for the DSST from our previous study,[18] and calculation of the amount of oxygen theoretically released from hemoglobin with a P50 of approximately 15 mmHg for stored erythrocytes, *versus* a P50 of 26.7 mmHg for fresh erythrocytes, at Po_2 40 mmHg (venous Po_2) using a within-subject design, a two-sided α of 0.05, and a power of 80% to detect a 20% improvement in DSST after transfusion. A second *a priori* power analysis, with a one-sided α of 0.05 and a power of 90% to detect a 25-ms decrease in reaction time after transfusion, provided results similar to that of the first power analysis.

Data are presented as mean with 95% confidence interval unless otherwise noted. Reaction time for a testing session was measured as the mean of all reaction times.

Comparisons for the Outcome Measures. Digit–symbol substitution test time, heart rate, and self-assessed energy level were performed within-day by analysis of variance with repeated measures, followed by the Tukey-Kramer test for multiple comparisons. Data for hemoglobin concentration, 2,3-DPG concentrations, and P50 were compared by *t* tests. Statistical significance was accepted at $P \leq 0.05$ for all tests.

We also tested for equivalence of the effect of transfusion of the fresh and stored erythrocytes. The confidence limits of the difference between the two types of cells for the difference in DSST at hemoglobin 5 and after transfusion (hemoglobin 7) were determined, and the probabilities of true effects being outside specified equivalence margins at 10 and 15 ms were calculated based on *t* tests comparing the estimated difference with the upper and lower equivalence margins, respectively.

Results

The volunteers were aged 23 (21–25) yr (mean and 95% confidence interval), were 1.74 (1.60–1.87) m tall, and weighed 64.0 (57.3–70.6) kg. There were six women and three men. Hemoglobin ($P = 0.69$), 2,3-DPG concentrations ($P = 0.60$), P50 ($P = 0.18$), and DSST times ($P = 0.60$) before hemodilution did not differ on the 2 experimental days (tables 1–3 and figs. 1–3). One of the volunteers did not follow directions correctly during the DSS tests. Consequently, his data for this variable were not usable, and we report the results of the other eight volunteers for the DSST and P50, but the results for all nine volunteers for all other variables, unless otherwise noted.

We reduced hemoglobin concentration to 5.4 (5.3–5.6) g/dl on the day that fresh erythrocytes were to be transfused and to 5.5 (5.4–5.6) g/dl on the day that old stored erythrocytes were to be transfused (not different between days, $P = 0.69$). Transfusion of fresh or stored erythrocytes increased the hemoglobin concentration equivalently, $P = 0.45$) to 7.4 (7.1–7.7) g/dl and 7.6 (7.2–8.0) g/dl, respectively (fresh, $P < 0.001$; stored, $P < 0.001$). These concentrations did not differ from those measured during production of acute isovolemic anemia (7.4, 7.1–7.6 g/dl and 7.5, 7.3–7.7 g/dl, respectively; fresh, $P = 0.84$; stored, $P = 0.62$). After transfusion of erythrocytes, the volunteers' arterial Po_2 values did not differ between the 2 experimental days (fresh: 94.9, 91–99 mmHg; stored: 94.1, 87–101 mmHg; $P = 0.85$).

Table 2. P50

Condition	Fresh	Stored	*P* Value
Baseline	27.7 (27.1–28.3)	27.4 (26.7–28.1)	0.18
7 g/dl hemoglobin, dilution	27.3 (26.5–28.0)	27.6 (26.8–28.5)	0.18
5 g/dl hemoglobin	27.2 (26.4–27.9)	27.6 (26.9–28.2)	0.10
Units transfused	25.0 (24.5–25.5)	15.0 (14.6–15.4)	< 0.001
7 g/dl hemoglobin after transfusion	27.3 (26.6–28.1)	24.8 (24.0–25.6)	< 0.001

Values are mean (95% confidence interval), in mmHg. All values are from blood withdrawn from the volunteers, except for those indicated as "units transfused," which are from those units of packed erythrocytes immediately before transfusion. P50 is an expression of the affinity of hemoglobin for oxygen; see text for complete definition. *P* is the probability of difference between fresh and stored. See text for additional statistical analyses.

Table 3. 2,3-Diphosphoglycerate Concentration

Condition	Fresh	Stored	*P* Value
Baseline	12.1 (10.1–14.0) [6]	11.9 (10.4–13.4) [6]	0.60
Units transfused	11.0 (10.0–11.9) [7]	1.1 (0.7–1.5) [8]	< 0.001

Values are mean (95% confidence interval), in μmol/g hemoglobin. Numbers in brackets are the number of subjects for which samples were tested. *P* is probability of difference between fresh and stored. See text for additional statistical analyses.

The time of storage for the fresh erythrocytes was 3.5 (3.1–4.0) h (median, 3.4 h; range, 2.3–4.9 h), and the time for the stored erythrocytes was 23 (21–25) days (median, 23 days; range, 18–29 days) ($P < 0.001$).

Primary Outcome Measure (DSST)

The DSST time did not differ on the 2 experimental days before production of acute isovolemic anemia (table 1; $P = 0.60$). Acute isovolemic anemia to 5 g/dl hemoglobin significantly increased (slowed) DSST time on both days (both $P < 0.001$; fig. 1) equivalently ($P = 0.62$ between days). Transfusion of erythrocytes stored for 3 weeks significantly decreased (sped) the DSST time ($P < 0.001$). Erythrocyte-induced decrease of DSST time did not differ between fresh and stored erythrocytes ($P = 0.96$; mean difference between the fresh and stored erythrocytes, 0.6 ms). The test of equivalence for effect of transfusion of fresh or stored erythrocytes on DSST (difference between values at hemoglobin 5 g/dl and at hemoglobin 7 g/dl after transfusion) indicated that the probabilities that the true numerical difference between erythrocyte storage types is more than 10 or 15 ms is 0.12 and < 0.05, respectively. The DSST times after transfusion of fresh or stored erythrocytes (7 g/dl hemoglobin concentration) did not differ from those at an equivalent hemoglobin concentration (7 g/dl) during production of anemia, before transfusion (fresh, $P = 0.23$; stored, $P = 0.88$).

Secondary Outcome Measures

Acute isovolemic anemia at a hemoglobin concentration of 5 g/dl increased the heart rate equivalently on the 2 days (fresh day: 65 [57–73] to 90 [81–99] beats/min, $P < 0.001$; stored day: 67 [61–73] to 93 [86–100] beats/min, $P < 0.001$; *P* between days at hemoglobin 5 = 0.34; fig. 3). Transfusion of fresh or stored erythrocytes equivalently ($P = 0.85$) reduced heart rate (fresh: by 10.7 [6.0–15.3] beats/min, $P < 0.001$; stored: by 10.3 [5.4–15.3] beats/min, $P = 0.0014$).

Acute isovolemic anemia at a hemoglobin concentration of 5 g/dl decreased the self-assessed energy level equivalently on the 2 days (fresh day: 7.6 [5.8–9.3] to 2.6 [1.5–3.8], $P < 0.001$; stored day: 6.6 [5.0–8.2] to 2.6 [1.2–3.9], $P < 0.001$; *P* between days at hemoglobin 5 = 0.96; fig. 4). Energy level did not differ between experimental days after transfusion of fresh or stored erythrocytes ($P = 0.80$).

Concentrations of 2,3-DPG and values of P50 did not differ on the 2 experimental days at baseline ($P = 0.60$

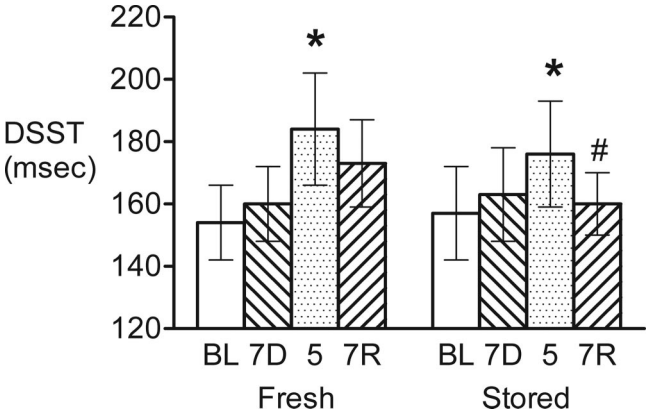

Fig. 1. Digit–symbol substitution test (DSST) on each experimental day, at baseline = 12 g/dl hemoglobin (BL; *white*), 7 g/dl hemoglobin (7D; *downward diagonals*), 5 g/dl hemoglobin (5; *stippled*), and after transfusion of either "fresh" or stored erythrocytes to return hemoglobin to 7 g/dl (7R; *upward diagonals*). DSST values are mean and 95% confidence interval for each response. * Acute isovolemic anemia to 5 g/dl hemoglobin increased response time on both days equivalently. # Erythrocytes stored for 23 (21–25) ± 4 days decreased DSST response time ($P = 0.023$) to a value not different from that at 7 g/dl hemoglobin during hemodilution ($P = 0.58$). n = 8.

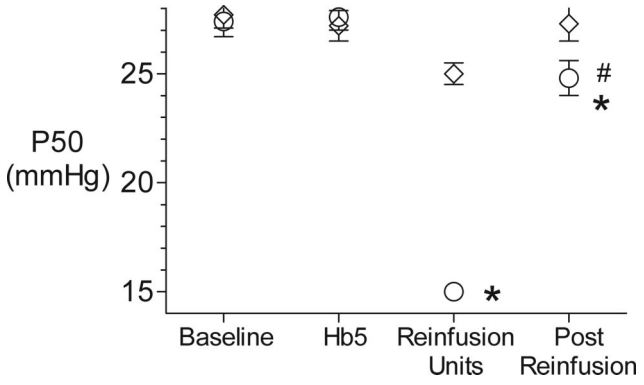

Fig. 2. Hemoglobin affinity for oxygen (P50) of the volunteers' blood, on each experimental day, at baseline = 12 g/dl hemoglobin, 5 g/dl hemoglobin (Hb5), of the transfused "fresh" (*diamonds*) or stored erythrocytes (*circles*) immediately before transfusion, and of the volunteers' blood after transfusion of either "fresh" or stored erythrocytes to return hemoglobin to 7 g/dl. Values are mean and 95% confidence interval. The 95% confidence interval does not appear for stored reinfusion units because the values fall within the boundaries of the symbol for the mean value. Acute isovolemic anemia to 5 g/dl hemoglobin did not alter P50 on either day. * Statistically significant difference between groups for reinfused erythrocytes ($P < 0.001$) and in the volunteers after transfusion ($P < 0.001$). # Statistically different from value at 5 g/dl hemoglobin ($P < 0.001$). n = 9.

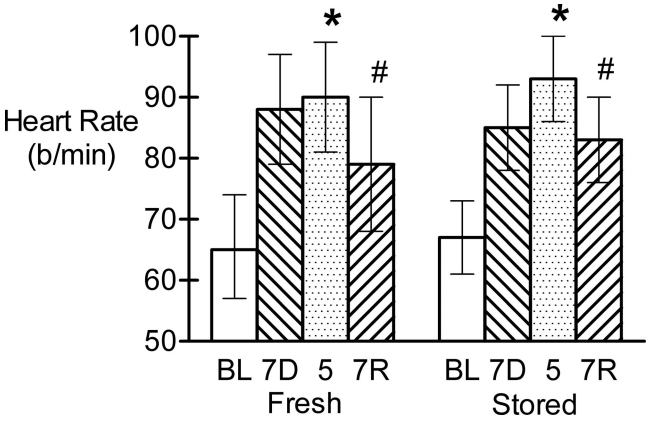

Fig. 3. Heart rate on each experimental day, at baseline = 12 g/dl hemoglobin (BL; *open*), hemoglobin 7 g/dl (7D; *downward diagonals*), 5 g/dl hemoglobin (5; *stippled*), and after transfusion of either "fresh" or stored erythrocytes to return hemoglobin to 7 g/dl (7R; *upward diagonals*). Values are mean and 95% confidence interval. * Acute isovolemic anemia to 5 g/dl hemoglobin increased heart rate on both days (fresh, $P < 0.001$; stored, $P < 0.001$) equivalently ($P = 0.34$). # Erythrocytes stored for 23 days decreased heart rate ($P < 0.001$) to a value not different from that at 7 g/dl hemoglobin during hemodilution ($P = 0.63$). n = 9.

and $P = 0.18$, respectively; table 1). Erythrocytes stored for 3 weeks had decreased concentrations of 2,3-DPG ($P < 0.001$; table 3) and decreased P50 (increased oxygen affinity of hemoglobin): at time of transfusion, 15.0 (14.6–15.4) mmHg *versus* at baseline, 27.4 (26.7–28.1) mmHg ($P < 0.001$; fig. 2 and table 2). Storage of erythrocytes for 3.5 h slightly decreased numerically (but not statistically significantly) 2,3-DPG concentrations (at the time of transfusion, 11.0 [10.0–11.9] μmol/g hemoglobin *vs.* at baseline, 12.1 [10.1–14.0] μmol/g; $P = 0.10$) and the

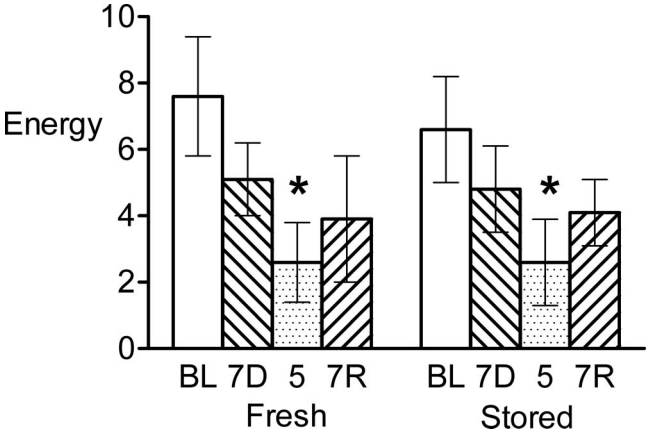

Fig. 4. Self-assessed energy level on each experimental day, at baseline = 12 g/dl hemoglobin (BL; *open*), 7 g/dl hemoglobin (7D; *downward diagonals*), 5 g/dl hemoglobin (5; *stippled*), and after transfusion of either "fresh" or stored erythrocytes to return hemoglobin to 7 g/dl (7R; *upward diagonals*). Values are mean and 95% confidence interval. * Acute isovolemic anemia to 5 g/dl hemoglobin decreased energy level on both days (fresh, $P < 0.001$; stored, $P < 0.001$) Energy levels did not differ after fresh *versus* stored erythrocyte transfusion ($P = 0.78$). n = 9.

hemoglobin affinity for oxygen (at time of transfusion, 25.0 [24.5–25.5] mmHg *vs.* at baseline, 27.7 [27.2–28.3] mmHg; $P < 0.001$; table 2), but less so than did storage for 3 weeks (for 2,3-DPG, P of difference $<$ 0.001; for P50, P of difference < 0.001; table 2). Therefore, transfusion of stored erythrocytes with depleted 2,3-DPG and decreased P50 (but not fresh erythrocytes) resulted in slightly decreased values for P50 in the circulating blood of volunteers after transfusion: P50: before transfusion, 27.6 (26.9–28.2); after transfusion, 24.8 (24.0–25.6); $P < 0.001$ (fig. 2 and table 2).

Discussion

Our primary finding reported here is that erythrocytes stored for 3 weeks are equally as efficacious as are fresh erythrocytes for reversing the effects of acute isovolemic anemia. This is the first prospective randomized trial in humans with a reproducible oxygen-dependent deficit to have tested the hypothesis that erythrocytes stored for at least 3 weeks, with a markedly increased hemoglobin affinity for oxygen (decreased P50), are as efficacious as are erythrocytes with hemoglobin with a normal affinity for oxygen (P50). The results reject the hypothesis that stored erythrocytes do not off-load oxygen in clinically meaningful quantities. We have confirmed our previous finding that acute isovolemic anemia slows human reaction time,[18,20] increases heart rate,[19,20,24] and decreases self-assessed energy level.[25] We showed previously that transfusion of erythrocytes stored for 4 h or less in CPDA-1 is efficacious in reversing the effects of acute anemia on reaction time,[18] heart rate,[18] and sense of energy.[25] We have now shown that although erythrocytes stored for 3 weeks in CPDA-1 are depleted of 2,3-DPG, resulting in a substantial decrease of P50, to 15 mmHg, nevertheless, they apparently release sufficient quantities of oxygen to reverse the deficits of acute anemia to an extent equivalent to that of erythrocytes with a normal P50 after being stored for less than 5 h.

The affinity of hemoglobin for oxygen increases when liquid blood is stored with preservatives.[16] 2,3-DPG is an important modulator of the interaction of hemoglobin and oxygen.[11–14] A decrease of 2,3-DPG concentration increases the affinity of hemoglobin for oxygen (decreases P50). During *ex vivo* storage of erythrocytes, 2,3-DPG concentrations and P50 decrease.[1,3] These observations have been confirmed repeatedly.[2,4–10,17] Blood stored for 14 days or more has a 2,3-DPG concentration of 1 μmol/g hemoglobin or less,[2,3,5,6,8–10] with a resultant hemoglobin P50 of 16–20 mmHg.[8,10,16] Hemoglobin with a normal P50 of 27 mmHg,[15] 97–98% saturated at normal arterial Po_2,[15,23] releases approximately 25% of its bound oxygen at the usual venous Po_2 of 40 mmHg. Hemoglobin with an increased affinity for oxy-

gen, such as that found in erythrocytes stored for more than 14 days, as was the case in our experiment, with a P50 of 15 mmHg, theoretically should release only approximately 2% of its bound oxygen at similar venous P_{O_2} and pH.

This has led some to propose that erythrocytes stored for several days or more are of limited or no efficacy in releasing oxygen[16]: the primary purpose for erythrocyte transfusion. Experiments in species other than man have produced mixed results. Methodologic issues and species differences make interpretation and application to humans difficult. Although a modest 2.4-mmHg decrease of P50 in man does not impair work performance,[26] studies in humans have suffered from not having produced or examined a state of inadequate oxygenation amenable to reversal. Demonstration of erythrocyte efficacy for oxygen delivery requires an ability to reliably and accurately measure an oxygenation deficit before and after transfusion. Previous studies in humans have been confounded by an inability to satisfy this requirement.[27–29] These studies have had other issues, as well, precluding an ability to draw conclusions regarding efficacy of stored erythrocytes. Gastric gradient for carbon dioxide was estimated in an unblinded exploratory study of patients in an intensive care unit; however, most patients did not meet the authors' criteria for gastric hypoxia,[28] and there are no accepted standards for hypoxia for this controversial methodology. Similarly, a recently published study designed to determine the feasibility of a full trial to examine clinical outcome after transfusion, although double-blinded and randomized, did not select patients for whom a deficit of oxygenation was demonstrated, and the difference in erythrocyte storage times, and the arbitrary sample size were likely insufficient to test the hypothesis.[29] Last, a study in septic patients, in whom an increase in measured oxygen consumption immediately after transfusion was not detected, could not provide an adequate test because the patients seemed to have normal oxygen consumption before transfusion and there was no measured deficit of oxygenation.[27] Furthermore, sepsis is not a good model to test improvement of delivery of usable oxygen owing to a deficit of oxygen uptake at the cellular level.[30,31] Therefore, even had there been an oxygenation deficit, it would have not been reversed by any erythrocyte transfusion. These thoughts are also supported by the lack of increase of oxygen consumption 6 h after transfusion in that study, when 60–80% of the 2,3-diphosphoglycerate should have been restored[2,5] with a theoretical increased release of oxygen from hemoglobin (if the hypothesis were valid). Our model, used in the study reported here, overcomes these difficulties, having shown both functional[18,20] and electroencephalographic[32] changes induced by inadequate oxygenation, that are reversible by increasing oxygenation by either breathing oxygen[20,32] or transfusion of fresh erythrocytes.[18]

In our study, the blood stored for less than 5 h had very small decreases in 2,3-DPG and P50. These minimal changes would seem to be in keeping with that reported previously.[33] However, the cells stored for 3 weeks had nearly depleted 2,3-DPG concentrations and a substantially reduced P50 (15 mmHg), also in accordance with values reported previously. The P50 of the stored blood that we report is slightly less than that reported by others (16–20 mmHg), possibly owing to our having applied a correction for base excess (approximately 1.5–2 mmHg) that may not have been applied by others. Despite these considerable changes and the theoretical consideration that the hemoglobin of these erythrocytes should be capable of releasing but a minimal amount of oxygen at usual physiologic venous values of P_{O_2}, P_{CO_2}, and pH, the stored erythrocytes were as efficacious as were the fresh cells in reversing the neurocognitive deficit and physiologic changes (heart rate and fatigue) caused by acute isovolemic anemia at a hemoglobin concentration of 5.5 g/dl.

The reason for the ability of the transfused stored erythrocytes to apparently provide quantities of oxygen at least equivalent to that of fresh erythrocytes is not immediately apparent. Several explanations are possible, although we do not have data either to support or to refute them. P50 is a descriptor of the physical–chemical function of hemoglobin and is defined as the P_{O_2} at which hemoglobin is 50% saturated with oxygen, at pH 7.4 and base excess 0. The relation between P_{O_2} and oxyhemoglobin saturation is affected by several parameters, including pH, temperature, base excess, and 2,3-DPG concentration. *In vivo* conditions, such as change of pH, can alter the *in vivo* relation between P_{O_2} and oxyhemoglobin saturation without affecting the intrinsic function of the hemoglobin molecule (the *in vitro* P50 value). It is possible that the acidosis within the erythrocyte shifted the hemoglobin–oxygen dissociation curve to the right, creating an *in vivo* condition of lesser affinity of hemoglobin for oxygen, thus reversing the effect of decreased 2,3-DPG concentration, as has been suggested by others.[26,34] We have calculated that at the mean measured *in vitro* pH of 6.787 and a base excess of −39.5 (at P_{CO_2} approximately 40 mmHg) of the blood stored for 3 weeks, at an oxyhemoglobin saturation of 50%, the P_{O_2} (in a sense, a functional, *in vivo* "P50") would have been approximately 33 mmHg, rather than the *in vitro* measured value for P50 of 15 mmHg. Hemoglobin with a P50 of 33 mmHg will release 38% of its bound oxygen at a venous P_{O_2} of 40 mmHg, rather than only 2% when the P50 is 15 mmHg. We have no data to indicate the duration for which the transfused erythrocytes remained severely acidotic. However, we measured a "mixed P50" of 24.8 mmHg, near the mathematical theoretical value of what was to be expected of the mixture of erythrocytes if the transfused erythrocytic intracellular pH were close to that of the recipient

plasma. Therefore, it seems that the acid that had accumulated within the erythrocytes during storage either may have been metabolized or may have moved into the plasma during the brief period between their transfusion and the time of testing (15–20 min). However, we have no data regarding this issue. Nor do we have data regarding transmembranal flux of other electrolytes after transfusion of stored erythrocytes that might have affected the intracellular environment and relation between oxygen and hemoglobin. Furthermore, if the *in vivo* "P50" were truly 33 mmHg, we should have measured an increased P50 after transfusion of the stored erythrocytes because, unlike when measured in stored blood, the *in vitro* determination of P50 of the recipients' blood did not require the large correction for pH.

It may also be possible that 2,3-DPG diffused from the recipients' erythrocytes into the transfused erythrocytes, thus increasing the P50 of the transfused hemoglobin. This would have the effect of converting the two *in vivo* populations of erythrocytes (see below: one with normal P50, and one with a greatly reduced P50) to a single population of erythrocytes of only slightly reduced P50. However, we have no data regarding this point.

A third possible explanation of our findings is that the stored erythrocytes, with reduced P50, altered cerebral blood flow sufficiently to reverse the oxygen delivery–dependent deficit induced by anemia. Cerebral blood flow increases with decreased arterial oxygen content.[35–43] However, if the anemia is sufficiently severe, that increase in cerebral blood flow is insufficient to compensate for the anemia,[37,44] as must have occurred in our subjects, with a proven oxygen delivery–induced cognitive function deficit. Cerebral blood flow also decreases in fetal lambs when their erythrocytes with low P50 fetal hemoglobin are replaced by adult sheep erythrocytes with normal P50,[45] and increases in rats after exchange transfusion with erythrocytes of decreased P50.[38] However, it has not been shown that decreasing hemoglobin P50 can further increase cerebral blood flow when the already increased cerebral blood flow (and likely at or near maximal) has been inadequate to preserve tissue oxygenation in response to anemia. For example, although calculated cerebral blood flow increases in anesthetized, hypovolemic, hyperventilated baboons, after transfusion with stored erythrocytes with a high affinity for oxygen, there was no alteration of cerebral oxygen consumption either before or after transfusion with either these or cells with a low affinity for oxygen.[46] Thus, there was no evidence that a state of inadequate oxygenation had been produced, and there existed no deficit to correct. It is not immediately apparent why theoretically further decreasing oxygen availability, by increasing the affinity of hemoglobin for oxygen, would increase cerebral blood flow not only in quantities to compensate for the decreased P50, but in excess, so that the anemia-induced deficit would be reversed as well. Furthermore, if such an increase were possible, it would imply that the mechanism governing the response of cerebral blood flow to reduced tissue oxygenation induced by altered P50 is different from and more potent than the mechanism (vasodilation, and perhaps decreased blood viscosity) regulating cerebral blood flow in response to decreased arterial oxygen content. This has not been demonstrated. Although the mechanisms responsible for increasing cerebral blood flow in response to anemia or hypoxia are incompletely understood and seem to overlap, but without complete concordance,[47,48] the few relevant data regarding cerebral blood flow during anemia and altered P50 seem to point to a common pathway.[38,45,49] Furthermore, increasing blood viscosity by increasing hematocrit, with a small decrease in P50 without changing arterial oxygen content, by transfusion of erythrocytes with nearly 100% methemoglobin, decreases cerebral blood flow and sagittal sinus Po_2 in unanesthetized lambs.[50] Therefore, adding erythrocytes containing hemoglobin with a decreased P50, while not altering the function or concentration of hemoglobin present before transfusion, likely decreased and not increased cerebral blood flow.

It has been suggested that the decreased ability of erythrocytes to deform, a property that is associated with decreased adenosine triphosphate concentrations with erythrocyte storage, decreases mean transfused erythrocyte *in vivo* survival secondary to impaired ability of erythrocytes to pass the microcirculation.[51,52] More recent data, using laser technology, have produced conflicting evidence regarding storage-induced decreased erythrocyte deformability and the associated hypothesis regarding impaired microcirculation and oxygen delivery.[53,54] Interestingly, rat erythrocytes with glutaraldehyde-produced decreased deformability are trapped by some tissues (spleen, lung, liver, and bone) but not by the brain, myocardium, or kidney.[55] Therefore, even if stored erythrocytes do have decreased ability to pass through some tissues, this may not be relevant to critical organs including the brain, as found by Simchon *et al.*[55] and supported by our results reported here.

It is appropriate to examine whether the methodology of this study was adequate to test the proposed hypothesis. First, our model is capable of testing the hypothesis because it reliably produces a testable oxygen delivery–dependent deficit. We have repeatedly demonstrated that acute isovolemic anemia at 5–6 g/dl hemoglobin decreases the speed of performance of the DSST[18,20] as it did in this study. In addition, we have shown that two independent methods of increasing tissue oxygenation, erythrocyte transfusion[18] and breathing oxygen,[20] reverse this deficit, whereas a placebo (breathing room air) does not.[18,20] Similarly, it might be suggested that transfusion of only 2 units of erythrocytes provided an insufficient test of the hypothesis. However, we tested the hypothesis with a reliable end point during a state of

oxygen-delivery dependency, and the amount of erythrocytes transfused was adequate to deliver sufficient amounts of oxygen so that both fresh and stored erythrocytes reversed completely the anemia-induced deficit.

Second, we have previously noted that heart rate is a reliable surrogate for the effects of acute isovolemic anemia, increasing 4 beats/min for each 1 g/dl decrease of hemoglobin concentration.[24] Heart rate in this study responded in a manner similar to that previously noted, and the increase with acute anemia was reversed equally by fresh and stored erythrocytes. It would seem unlikely that the undefined "sensor" of acute decrease of hemoglobin concentration that causes an increase in heart rate would have responded to a hemoglobin that did not release oxygen. The decrease in heart rate with transfusion of stored erythrocytes provides further support for our primary finding of the reversal of the neurocognitive deficit.

Third, after transfusion of stored blood, the mean P50 of the circulating hemoglobin decreased only to 24.8 mmHg. This might seem to suggest to some that at this P50, there would be no reason to infer a deficit of dissociation of oxygen from hemoglobin. Woodson *et al.*,[56] through metabolic alterations, noted that a similar decrease of P50 did not alter human work performance. However, the value for P50 that we found after transfusion of stored erythrocytes is misleading. This value represents a mixture of two different populations of erythrocytes, and not a single mean with a normal distribution. The circulating erythrocytes present before transfusion had a P50 of 27.6 mmHg and accounted for an estimated 72.4% of the erythrocytes present after transfusion of erythrocytes with a P50 of 15.0 mmHg. The P50 of the former cells would not have changed after transfusion, and that of the latter cells would have changed exceedingly little, if at all, in the 15–20 min after transfusion during which the DSST was administered. Erythrocytes that are depleted of 2,3-DPG, when transfused, increase their 2,3-DPG concentration at a rate less than 0.2 μmol/g hemoglobin/h.[5] Several problems preclude an exact calculation of a value for what should have been the theoretical "mean" P50 of the mixture of native and transfused stored erythrocytes. However, the value of 24.8 mmHg that we determined seems to be within a reasonable expectation of the mixture of erythrocytes with differing hemoglobin affinities for oxygen. Therefore, at the time of DSS testing, the circulating hemoglobin in the subjects consisted of a hemoglobin concentration of 5.5 g/dl with normal P50 (27.6 mmHg), combined with a hemoglobin concentration of 2.0 g/dl with a greatly increased affinity for oxygen (low P50, 15.0 mmHg), proving an adequate test of the hypothesis.

Fourth, it is possible that even the very small amount of oxygen theoretically released by hemoglobin with a P50 of 15 mmHg would be sufficient to reverse the neurocognitive deficit and heart rate increase found in this study. This seems unlikely, because both were reversed to the same full extent as that noted by transfusion of similar quantities of erythrocytes with a P50 of 25 mmHg, which would release quantities of oxygen similar to that of hemoglobin with a normal P50 of 27 mmHg. The amount of oxygen theoretically released by the stored cells should have added the equivalent of approximately 0.2 g hemoglobin/dl (one tenth of the transfused 2.0 g/dl), resulting in a total functional hemoglobin concentration of less than 6 g/dl, a value at which we have previously noted this same neurocognitive deficit.[18]

Fifth, one may suggest that the reversal of the noted effects of anemia was a result of the small augmentation of blood volume, by transfusion of approximately 250 ml of erythrocytes, and not a result of augmented tissue oxygenation. It is very unlikely that the subjects were hypovolemic when tested during acute anemia. Both considerations of the pharmacokinetics of infused human albumin[21] and experimental data in humans[19] provide support that our volume replacement paradigm maintained normovolemia. We did not test whether the likely very mild hypervolemia produced by erythrocyte transfusion would similarly reverse the acute anemia-induced changes. This potential mechanism seems unlikely for three reasons: (1) The mild augmentation of blood volume by asanguinous fluid or nonefficacious hemoglobin would produce further dilution of the recipients' erythrocytes and a further accentuated functional anemia; this unlikely possibility could have been tested by transfusion blood with a hemoglobin concentration of similar to that measured during acute anemia, 5.5 g/dl, but we did not do that. (2) Such a thesis implies that increasing cardiac output or cerebral blood flow despite further aggravation of the severe anemia would be beneficial. We have previously demonstrated that progressive anemia at this level is insufficiently compensated by increased cardiac output. It is not known whether further anemia at this level would increase cerebral blood flow in conscious humans, and even if it did, it would be exceedingly unlikely that cerebral blood flow would increase out of proportion to the produced anemia, increasing cerebral oxygen delivery and reversing the inadequate cerebral oxygen delivery that existed at a higher hemoglobin concentration, while having no such deficit at a lower concentration. (3) Our previous experiments with breathing oxygen or air at similar hemoglobin concentrations in similar volunteers clearly indicated that increasing oxygen concentration (and presumably delivery) without infusion of any additional fluids (and thus no alteration of blood volume) similarly reversed the same neurocognitive deficit.

Sixth, it might be possible that neither type of erythrocytes augmented oxygen delivery to the brain: that our results were caused by an adaptation during the brief period between the test at the nadir hemoglobin con-

centration and the test after transfusion to return hemoglobin concentration to 7 g/dl. In a previous study, similar volunteers were given to breathe, at a similar nadir hemoglobin concentration, in random order, either air or oxygen, followed by the alternative gas.[20] If time were a factor, there would have been a significant difference in the effect of order; there was not. This suggests that time and adaptation did not contribute to the results in the few minutes between the two tests of neurocognitive function.

Last, we studied a small number of subjects. However, the sample size was that determined by an *a priori* power analysis and confirmed by a second, independent *a priori* power analysis. Furthermore, we are constrained, as are others, in the conduct of human experimentation, to study the minimum number of people required. Our clear results confirm the lack of necessity of having studied a larger population.

Our results have clinical implications. By using the only reproducible model in humans that reliably shows a readily measured reversible deficit of acute isovolemic anemia, we have demonstrated that erythrocytes stored in the standard clinical manner are as efficacious as are fresh erythrocytes in providing oxygen to tissues. Therefore, it does not seem warranted to require fresh rather than stored erythrocytes for this purpose, which is the intent of erythrocyte transfusion.[57]

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References

1. Chanutin A: The effect of the addition of adenine and nucleosides at the beginning of storage on the concentrations of phosphates of human erythrocytes during storage in acid-citrate-dextrose and citrate-phosphate-dextrose. Transfusion 1967; 7:120–32

2. Beutler E, Wood L: The *in vivo* regeneration of red cell 2,3 diphosphoglyceric acid (DPG) after transfusion of stored blood. J Lab Clin Med 1969; 74:300–4

3. Beutler E, Meul A, Wood L: Depletion and regeneration of 2,3-diphosphoglyceric acid in stored red blood cells. Transfusion 1969; 9:109–15

4. Dawson RB Jr, Kocholaty WF, Gray JL: Hemoglobin function and 2,3-DPG levels of blood stored at 4 C in ACD and CPD: pH effect. Transfusion 1970; 10:299–304

5. Heaton A, Keegan T, Holme S: *In vivo* regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-1, AS-3 and CPDA-1 red cells. Br J Haematol 1989; 71:131–6

6. Moore G, Peck CC, Sohmer P, Zuck TF: Some properties of blood stored in anticoagulant CPDA-1 solution: A brief summary. Transfusion 1981; 21:135–7

7. Moore G, Ledford M, Peck CC: The *in vitro* evaluation of modifications in CPD-adenine anticoagulated-preserved blood at various hematocrits. Transfusion 1980; 20:419–26

8. Sohmer P, Moore EE, Beutler E, Peck CC: *In vivo* viability of red blood cells stored in CPDA-2. Transfusion 1982; 22:479–84

9. Vora S, West C, Beutler E: The effect of additives on red cell 2,3 diphosphoglycerate levels in CPDA preservatives. Transfusion 1989; 29:226–9

10. Valeri CR, Valeri DA, Gray A, Melaragno A, Dennis RC, Emerson CP: Viability and function of red blood cell concentrates stored at 4 degrees C for 35 days in CPDA-1, CPDA-2, or CPDA-3. Transfusion 1982; 22:210–6

11. Benesch R, Benesch RE: Intracellular organic phosphates as regulators of oxygen release by haemoglobin. Nature 1969; 221:618–22

12. Benesch R, Benesch RE, Yu CI: Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin. Proc Natl Acad Sci U S A 1968; 59:526–32

13. Chanutin A, Curnish R: Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch Biochem Biophys 1967; 121: 96–102

14. Benesch R, Benesch RE: The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. Biochem Biophys Res Comm 1967; 26:162–7

15. Severinghaus J: Simple, accurate equations for human blood O2 dissociation computations. J Appl Physiol 1979; 46:599–602

16. Valtis DJ, Kennedy AC: Defective gas-transport function of stored red blood-cells. Lancet 1954; 266:119–24

17. Valeri CR, Hirsch N: Restoration *in vivo* of erythrocyte adenosine triphosphate, 2,3-diphosphoglycerate, potassium ion, and sodium ion concentrations following the transfusion of acid-citrate-dextrose-stored human red blood cells. J Lab Clin Med 1969; 73:722–33

18. Weiskopf RB, Kramer JH, Viele M, Neumann M, Feiner J, Watson JJ, Hopf H, Toy P: Acute severe isovolemic anemia impairs cognitive function and memory and humans. ANESTHESIOLOGY 2000; 92:1646–52

19. Weiskopf RB, Viele M, Feiner J, Kelley S, Lieberman J, Noorani M, Leung J, Fisher D, Murray W, Toy P, Moore M: Human cardiovascular and metabolic response to acute, severe isovolemic anemia. JAMA 1998; 279:217–21

20. Weiskopf R, Feiner J, Hopf HW, Viele M, Watson J, Kramer JH, Ho R, Toy P: Oxygen reverses deficits of cognitive function and memory and increased heart rate induced by acute severe isovolemic anemia. ANESTHESIOLOGY 2002; 96:871–7

21. Payen J-F, Vuillez J-P, Geoffray B, Lafond J-L, Comet M, Stieglitz P, Jacquot C: Effects of preoperative intentional hemodilution on the extravasation rate of albumin and fluid. Crit Care Med 1997; 25:243–8

22. Ellis R: Determination of PO2 from saturation (letter). J Appl Physiol 1989; 67:902

23. Roughton F, Severinghaus J: Accurate determination of O2 dissociation curve of human blood above 98.7% saturation with data on O2 solubility in unmodified human blood from 0°C to 37°C. J Appl Physiol 1973; 35:861–9

24. Weiskopf R, Feiner J, Hopf HW, Viele M, Watson J, Lieberman J, Kelley S, Toy P: Heart rate increases linearly in response to acute isovolemic anemia. Transfusion 2003; 43:235–40

25. Toy P, Feiner J, Viele M, Watson J, Yeap H, Weiskopf R: Fatigue during acute isovolemic anemia in healthy resting humans. Transfusion 2000; 40:457–60

26. Wranne B, Nordgren L, Woodson R: Increased blood oxygen affinity and physical work capacity in man. J Clin Lab Invest 1974; 33:347–52

27. Marik P, Sibbald W: Effect of stored-blood transfusion on oxygen delivery in patients with sepsis. JAMA 1993; 269:3024–9

28. Walsh T, McArdle F, SA M, Maciver C, Maginnis M, Prescott R, McClelland D: Does the storage time of transfused red blood cells influence regional or global indexes of tissue oxygenation in anemic critically ill patients? Crit Care Med 2004; 32:364–71

29. Hebert P, Chin-Lee I, Fergusson D, Blajchman M, Martineau R, Clinch J, Olberg B: A pilot trial evaluating the clinical effects of prolonged storage of red cells. Anesth Analg 2005; 100:1433–8

30. Astiz M, Rackow EC, Weil MH, Schumer W: Early impairment of oxidative metabolism and energy production in severe sepsis. Circ Shock 1988; 26:311–20

31. Morelli A, Rocco M, Conti G, Orecchioni A, De Gaetano A, Coluzzi F, Vernaglione E, Pelaia P, Pietropaoli P: Effects of short-term fenoldopam infusion on gastric mucosal blood flow in septic shock. ANESTHESIOLOGY 2004; 101:576–82

32. Weiskopf R, Toy P, Hopf HW, Feiner J, Finlay H, Takahashi M, Bostrom A, Songster C, Aminoff MJ: Acute isovolemic anemia impairs central processing as determined by P300 latency. Clin Neurophysiol 2005; 116:1028–32

33. Hogman C, Knutson F, Loof H: Storage of whole blood before separation: the effect of temperature on red cell 2,3 DPG and the accumulation of lactate. Transfusion 1999; 39:492–7

34. Woodson R, Wranne B, Detter J: Effect of increased blood oxygen affinity on work performance of rats. J Clin Invest 1973; 52:2717–24

35. Borgstrom L, Johannsson H, Siesjo B: The influence of acute normovolemic anemia on cerebral blood flow and oxygen consumption of anesthetized rats. Acta Physiol Scand 1975; 93:505–14

36. Johannsson H, Siesjo B: Blood flow and oxygen consumption in the rat brain in dilutional anemia. Acta Physiol Scand 1974; 91:136–8

37. Johannsson H, Siesjo B: Brain energy metabolism in anesthetized rats in acute anemia. Acta Physiol Scand 1975; 93:515–24

38. Woodson R, Auerbach S: Effect of increased oxygen affinity and anemia on cardiac output and its distribution. J Appl Physiol 1982; 53:1299–306

39. Brown MM, Wade JP, Marshall J: Fundamental importance of arterial oxygen content in the regulation of cerebral blood flow in man. Brain 1985; 108:81–93

40. Hirakata H, Yao H, Osato S, Ibayashi S, Onoyama K, Otsuka M, Ichiya Y, Kuwabara Y, Masuda Y, Masatoshi F: CBF and oxygen metabolism in hemodialysis patients: Effects of anemia correction with recombinant human EPO. Am J Physiol 1992; 262:F737–43

41. Michenfelder J, Theye R: The effects of profound hypocapnia and dilutional anemia on canine cerebral metabolism and blood flow. ANESTHESIOLOGY 1969; 31:449–57

42. Cohen PJ, Alexander SC, Smith TC, Reivich M, Wollman H: Effects of hypoxia and normocarbia on cerebral blood flow and metabolism in conscious man. J Appl Physiol 1967; 23:183–9

43. Ulatowski J, Bucci E, Razynska A, Traystman RJ, Koehler RC: Cerebral blood flow during hypoxic hypoxia with plasma-based hematocrit hemoglobin at reduced hematocrit. Am J Physiol 1998; 274:H1933–42

44. Morimoto Y, Mathru M, Martinez-Tica J, Zornow M: Effects of profound anemia on brain tissue oxygen tension, carbon dioxide tension, and pH in rabbits. J Neurosurg Anesthesiol 2001; 13:33–9

45. Rosenberg A, Harris A, Koehler R, Hudak M, Traystman R, Jones M Jr: Role of O2-hemoglobin affinity in the regulation of cerebral blood flow in fetal sheep. Am J Physiol 1986; 251:H56–62

46. Valeri CR, Rorth M, Zaroulis G, Jakubowski M, Vescera S: Physiologic effects of transfusing red blood cells with high or low affinity for oxygen to passively hyperventilated, anemic baboons: Systemic and cerebral oxygen extraction. Ann Surg 1975; 181:106–13

47. Todd MM, Farrell S, Wu B: Cerebral blood flow during hypoxemia and hemodilution in rabbits: Different roles for nitric oxide? J Cereb Blood Flow Metab 1997; 17:1319–25

48. Tomiyama Y, Brian JJ, Todd M: Plasma viscosity and cerebral blood flow. Am J Physiol Heart Circ Physiol 2000; 279:H1949–54

49. Koehler RC, Traystman RJ, Jones M Jr: Influence of reduced oxyhemoglobin affinity on cerebrovascular response to hypoxic hypoxia. Am J Physiol 1986; 251:H756–63

50. Hudak M, Koehler RC, Rosenberg A, Traystman RJ, Jones MJ: Effect of hematocrit on cerebral blood flow. Am J Physiol 1986; 251:H63–70

51. Card RT, Mohandas N, Mollison PL: Relationship of post-transfusion viability to deformability of stored red cells. Br J Haematol 1983; 53:237–40

52. La Celle P: Alteration of deformability of the erythrocyte membrane in stored blood. Transfusion 1969; 9:238–45

53. Izzo P, Manicone A, Spagnuolo A, Lauta V, Di Pasquale A, Di Monte D: Erythrocytes stored in CPD SAG-mannitol: Evaluation of their deformability. Clin Hemorheol Microcirc 1999; 21:335–9

54. Raat NJ, Verhoeven A, Mik E, Gouwerok C, Verhaar R, Goedhart P, de Korte D, Ince C: The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. Crit Care Med 2005; 33:39–45

55. Simchon R, Jan K-M, Chien S: Influence of reduced red cell deformability on regional blood flow. Am J Physiol 1987; 253:H898–903

56. Woodson R, Wills R, Lenfant C: Effect of acute and established anemia on O_2 transport at rest, submaximal and maximal work. J Appl Physiol 1978; 44:36–43

57. American Society of Anesthesiologists Task Force on Blood Component Therapy: Practice guidelines for blood component therapy: A report by the American Society of Anesthesiologists Task Force on Blood Component Therapy. ANESTHESIOLOGY 1996;84:732–47