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Acute Normovolemic Hemodilution Improves Oxygenation in Ischemic Flap Tissue

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Background: **The flaps used in reconstructive surgery are prone to ischemia and hypoxia, which imply a considerable risk of wound-healing complications. During normovolemic hemodilution, the oxygenation may further deteriorate because of the lack of erythrocytes or improve because of increased microcirculatory blood flow. The aim of this study was to investigate the net effect of normovolemic hemodilution of various degrees on the microcirculation and oxygenation in ischemic flap tissue in adult minipigs.**

Methods: **A rectangular flap was raised in the middle of the epigastrium, consisting of an adequately perfused portion and a partly ischemic portion. The animals were randomly assigned to either the control group (n = 10) or the experimental group (n = 10) receiving graded normovolemic hemodilution with 6% hydroxyethyl starch 200–0.5.**

Results: **Normovolemic hemodilution caused a significant linear increase in total blood flow to the flap (measured by transit time flowmetry). In the ischemic flap tissue, both oxygen tension (measured by polarographic cells) and venous base excess were transiently improved during hemodilution (F = 4.79 and *P* = 0.019 for the regression of tissue oxygen tension on hemoglobin and hemoglobin squared, and F = 4.18 and *P* = 0.029 for base excess). The expected values reached a peak at hemoglobin concentrations of 9.1 and 8.5 g/dl, respectively. The measured values at this level of hemodilution were 17 ± 10.7 mmHg (mean ± SD) *versus* 7.6 ± 1.9 mmHg (baseline) for oxygen tension and −1.7 ± 3.4 *versus* −5.6 ± 4.1 mM for venous base excess.**

Conclusions: **Our results suggest that the oxygenation in ischemic and hypoxic flap tissue may be improved by normovolemic hemodilution. The maximal benefit may be expected at a hemoglobin concentration at or slightly less than 9 g/dl.**

DURING the past decades, the risks associated with allogeneic blood transfusions have led to a more restrictive management of erythrocyte substitution after traumatic and surgical blood loss.[1] This concept was supported by a large number of reports documenting that hemodilution as a result of resuscitation with crystalloid or colloid solutions is well tolerated in healthy organisms, provided normovolemia and normotension were maintained.[1,2] Despite the lack of oxygen carriers, adequate oxygen delivery to the tissues is achieved by an increase in cardiac output. On the capillary level, normovolemic hemodilution causes an acceleration of erythrocyte velocity because of a reduction of blood viscosity.[3] In addition, the microcirculatory hematocrit can be maintained at a higher level compared with systemic hematocrit.[4,5] The product of hematocrit and erythrocyte velocity determines the erythrocyte flux, which reaches its maximum at a hematocrit between 30 and 35%.[3,5] At this level of hemodilution, the capacity to transport oxygen has been reported to be maximal.[6] Such levels of hemodilution have therefore been used clinically to improve tissue oxygenation in a variety of ischemic disorders related to peripheral vascular diseases.[7,8]

On the other hand, it has been postulated that the lack of oxygen carriers during hemodilution may cause hypoxic damage in jeopardized tissues, such as surgical flaps.[9] It has been demonstrated that a large amount of oxygen may exit into the tissue on an arteriolar level, especially during low-flow conditions.[10] It may therefore be assumed that the diluted blood may not be capable of maintaining a sufficient content of oxygen when reaching the extended flap tissue, which is perfused *via* a collateral vascularization.

Wound-healing complications, such as wound dehiscence, infection, and tissue necrosis, have been attributed to cell hypoxia.[11] However, there is a paucity of scientific data documenting the effect of normovolemic hemodilution on the oxygenation in ischemic flap tissue. On one hand, it may be hypothesized that this tissue would experience an improved oxygenation caused by an increased perfusion. On the other hand, the reduction of oxygen carriers may result in a reduced amount of oxygen reaching the extended microvasculature.

The aim of the current study was to analyze the net effect of normovolemic hemodilution of various degrees on the oxygenation in ischemic flap tissue in a large animal model most closely simulating clinical conditions. For this purpose, the recently described porcine myocutaneous abdominal flap model was used.[12] The flap consists of an anatomically perfused part and an extended part, which is rendered ischemic because of interruption of its anatomic blood supply, thus being perfused *via* a collateral vascular system. The model allows for simultaneous assessment of total blood flow to the flap, local microcirculatory blood flow, and tissue oxygenation in both the anatomically perfused and the ischemic parts. Furthermore, blood samples can be obtained from the venous effluent of both parts without interfering with their venous drainage. Because of its widespread use, hydroxyethyl starch was chosen as the diluting colloid.

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Materials and Methods

The study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and with the approval of the Animal Care Committee of the County of Berne, Switzerland. Twenty-three minipigs (body weight, 26–44 kg) were assigned by their identification number to either the control group (n = 11) or the experimental group (n = 12) receiving graded normovolemic hemodilution with 6% hydroxyethyl starch 200–0.5 (Isohaes; Fresenius, Stans, Switzerland).

General anesthesia was induced with 10 mg/kg body weight ketamine given intramuscularly, followed 10 min later by 5 mg/kg metomidate, 0.05 mg/kg atropine, and 2 mg/kg azaperone given intravenously for tracheal intubation. Anesthesia was maintained with halothane and 79% nitrous oxide in oxygen. Inhaled and exhaled concentrations of halothane and nitrous oxide were continuously monitored with a multigas analyzer (Hellige SMU 611; Hellige AG, Freiburg, Germany). The animals received volume-controlled ventilation with a positive end-expiratory pressure of 5 cm H_2O (Tiberius 19; Drägerwerk AG, Lübeck, Germany). Tidal volume was kept at 10 ml/kg body weight and respiratory rate (13–18 breaths/min) was adjusted to maintain alveolar carbon dioxide tension constant. The body and flap temperatures were kept constant with a warming mattress and patient air-warming system (WarmTouch 5700; Mallinckrodt, Hennef, Germany). Ringer's lactate was administered intravenously at a rate of 10–15 ml $\cdot$ kg^{-1} $\cdot$ h^{-1} during surgery and 5 ml $\cdot$ kg^{-1} $\cdot$ h^{-1} thereafter.

Animal Preparation

The left jugular vein and carotid artery were cannulated, the pulmonary artery (thermodilution catheter; Arrow, Reading, PA) and central venous catheters were inserted *via* the left femoral vein, and the urinary bladder was catheterized through a small laparotomy. A myocutaneous flap was raised in the epigastrium as described previously (fig. 1).[12] A rectangular, transverse skin paddle measuring 16 × 8 cm was designed in the epigastrium, centered in the midline. The superficial epigastric veins were cannulated on each side of the flap to obtain blood samples. The entire left contralateral part of the skin paddle was raised on an epifascial plane. On the right ipsilateral side, the lateral 4 cm of the flap was dissected in the same way, whereas the medial portions were left attached to the anterior rectus sheath in an area of 8 × 4 cm. The anterior rectus sheath and the rectus abdominis muscle around this area were incised. The caudal border of the muscle was transected and elevated from the posterior rectus sheath. After visualizing the superior epigastric vessels on the undersurface of the rectus muscle, the cephalad border of the muscle was divided and the pedicle dissected free. The entire flap was completely detached from the surrounding tissue and merely pedicled on the superior epigastric vessels. Finally, the flap was sutured into its original place.

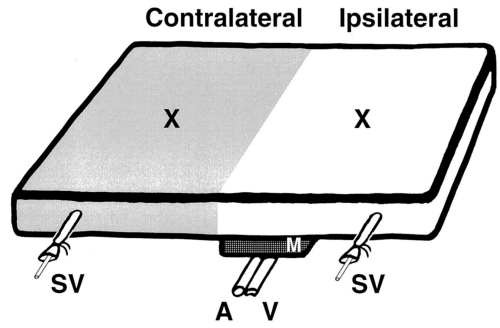

Fig. 1. Schematic diagram of the flap model. The flap is pedicled on the right superior epigastric artery (A) and vein (V), which give off branches perforating the rectus abdominis muscle (M), supplying the ipsilateral part of the flap. The contralateral part of the flap is perfused indirectly *via* connecting vessels to the ipsilateral part. The superficial epigastric veins (SV) are cannulated for sampling the venous blood of each part of the flap. The middle of each part of the flap (X) is where microcirculatory blood flow and tissue oxygen tension were measured. Total blood flow to the flap was measured in the right superior epigastric artery.

Hemodynamic Monitoring

Arterial blood pressure, central venous pressure, mean pulmonary artery pressure, and pulmonary capillary wedge pressure were recorded with a quartz pressure transducer (129A; Hewlett-Packard, Andover, MA) and displayed continuously on a multimodular monitor (Hellige SMU 611; Hellige AG) and recorder (Hellige SMR 821; Hellige AG). Heart rate was obtained from an electrocardiograph. Cardiac output was measured by a thermodilution technique and averaged over four consecutive measurements (cardiac output module, Hellige SMU 611; Hellige AG). Central venous blood temperature was recorded from the thermistor in the pulmonary artery catheter.

Respiratory Monitoring

Expired minute volume, tidal volume, respiratory rate, and end-expiratory peak and inspiratory pressure, inspired and end-tidal carbon dioxide concentrations, inspired and expired oxygen concentrations, and end-tidal halothane concentrations were monitored continuously throughout the experiment. Samples for arterial blood gas analysis were drawn from the carotid artery catheter and analyzed immediately (ABL 625; Radiometer, Copenhagen, Denmark).

Transit Time Flowmetry

Total blood flow to the flap was measured continuously with the use of ultrasonic transit time flowmetry (HT 206 flowmeter; Transonic Systems Inc., Ithaca, NY).[13] The signal was obtained from a 2-mm probe, which was placed around the pedicle artery. The transit

time ultrasound volume flowmeter has been demonstrated to provide adequate measures of arterial and venous flows in experimental animals if carefully positioned and aligned.[14] In particular, the validity of the signal was not influenced by changes in vascular diameter and hematocrit.

Laser Doppler Flowmetry

Microcirculatory blood flow was monitored continuously with a multichannel laser Doppler flowmeter system (The Oxford Array; Oxford Optronix, Oxford, United Kingdom). The suturable miniature surface probes used (SP 300; Oxford Optronix) were designed to measure microcirculatory blood flow (perfusion units) to a depth of 1 mm. Because of a high intersite variability,[13,15] the data are given in percentages of baseline. The probes were sutured to the intact abdominal skin (control skin) and the center of the ipsilateral and contralateral parts of the flap.

Tissue Oxygen Tension

Partial tissue oxygen tension was assessed with polarographic microprobes (Revoxode CC1; GMS GmbH, Kiel, Germany). The method relies on the principle of a Clark-type microcell. The sample area lies within 1 mm of the cell, which has a length of 5 mm. According to the manufacturer, the probes allow for continuous readings without recalibration for several consecutive days. Before each experiment, the probes were checked by exposing them to room air and an oxygen-free solution provided by the manufacturer. Tissue oxygenation was measured at the same location as microcirculatory blood flow by inserting the probes into the subcutaneous tissue at a subdermal plane.

The data for transit time, laser Doppler flowmetry, and tissue oxygen tension were acquired online *via* a multichannel interface (Mac Paq MP 100; Biopac Systems Inc., Goleta, CA) with acquisition–analysis software (Acqknowledge 3.0; Biopac Systems Inc.) to a portable computer (Macintosh Powerbook 180C; Apple Computer Inc., Cupertino, CA). The values given represent averages taken over observation periods of 5 min.

Experimental Protocol

After completion of the flap dissection, hemodynamic and respiratory variables were allowed to stabilize for 1 h before baseline measurements were taken. Animals in the experimental group then underwent graded hemodilution by stepwise withdrawal of blood and isovolemic replacement with 6% hydroxyethyl starch 200–0.5 every 30 min. The amount of blood exchange was chosen to obtain a drop of the hemoglobin concentration of 1 g/dl during each step of hemodilution. The measurements were taken immediately before the next blood exchange and until a hemoglobin concentration of 5 g/dl, because some animals tended to decompensate hemodynamically during further hemodilution in terms of developing severe arterial hypotension. At the end of the experiments, the animals were killed with an intravenous injection of potassium chloride. Animals were excluded from this study if the surgical blood loss exceeded 5% of the total blood volume and if hyperthermia with a core temperature greater than 40°C occurred.

Statistical Analysis

All data are given as mean ± SD. For the systemic data, paired analysis of variance followed by Bonferroni posttest were used to assess the differences for repeat measures. $P < 0.05$ represented statistical significance.

The data on transit time flowmetry, laser Doppler flowmetry, tissue oxygen tension, and base excess were analyzed by multiple regression analysis, treating observations on the same animal as clusters. It was necessary to perform a separate analysis for each site and response. Factors included in each regression were animal effects, hemoglobin concentration, and, if appropriate, hemoglobin squared. This method of analysis allowed a sparse yet flexible modeling of the dependence of each response on hemoglobin and thus permitted identification of optimal conditions. The dependence of each response on hemoglobin and hemoglobin squared was expressed by *P* and F, respectively.

Results

At baseline, the core temperature was 37.8 ± 1.1°C in the control group and 37.6 ± 0.9°C in the hemodilution group. The end-tidal halothane concentration was 0.57 ± 0.14% in the control group and 0.63 ± 0.19% in the hemodilution group, and the alveolar carbon dioxide tension was 37 ± 3 and 35 ± 5 mmHg, respectively. These values remained virtually unchanged throughout the experiment in both groups.

Hematologic, respiratory, and hemodynamic data are summarized in tables 1 and 2. The stepwise hemodilution was reflected by a gradual decrease in hemoglobin concentration and hematocrit from 12.2 ± 0.5 to 4.9 ± 0.4 g/dl ($P < 0.01$) and from 38 ± 3 to 16 ± 3% ($P < 0.01$), respectively. The base excess decreased from 3.7 ± 1.5 to 2.0 ± 1.8 mM after hemodilution to a hemoglobin concentration of 5 g/dl ($P < 0.05$). The hemodilution was paralleled by a gradual increase in arterial oxygen tension (not significant). Furthermore, hemodilution resulted in a gradual increase in cardiac output from 4.5 ± 0.9 to 6.9 ± 2.2 l/min ($P < 0.01$) and in heart rate from 94 ± 13 to 117 ± 17 beats/min ($P < 0.01$). No significant changes were observed for the other parameters.

The gradual hemodilution caused a linear increase in total blood flow to the flap ($P = 0.003$) from 10.4 ± 3.6 to 16.2 ± 5.4 ml/min (fig. 2).

Table 1. Hematologic and Respiratory Data

	Baseline	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Hemoglobin concentration (g/dl)								
Control	12.2 ± 1.5	12.4 ± 1.5	12.2 ± 1.6	12.4 ± 1.7	12.5 ± 1.6	12.5 ± 1.3	12.6 ± 1.5	12.7 ± 1.8
Hemodilution	12.2 ± 0.5	10.9 ± 0.4†	10.1 ± 0.4†	9.1 ± 0.2†	8.1 ± 0.2†	6.9 ± 0.2†	6.0 ± 0.3†	4.9 ± 0.4†
Hematocrit (%)								
Control	37 ± 5	38 ± 5	37 ± 5	38 ± 5	38 ± 5	38 ± 4	39 ± 4	39 ± 5
Hemodilution	38 ± 3	35 ± 2*	32 ± 2†	29 ± 2†	26 ± 2†	22 ± 1†	20 ± 1†	16 ± 3†
Blood exchange (% total blood volume)								
Control	0	0	0	0	0	0	0	0
Hemodilution	0	11 ± 3†	18 ± 6†	26 ± 3†	33 ± 4†	42 ± 4†	49 ± 4†	59 ± 4†
Arterial oxygen tension (mmHg)								
Control	98 ± 9	97 ± 11	99 ± 9	98 ± 10	98 ± 10	96 ± 12	98 ± 11	99 ± 14
Hemodilution	101 ± 10	102 ± 8	107 ± 10	102 ± 12	104 ± 14	104 ± 21	107 ± 15	109 ± 11
Arterial carbon dioxide tension (mmHg)								
Control	37 ± 4	38 ± 4	38 ± 4	38 ± 4	38 ± 3	38 ± 3	38 ± 3	36 ± 4
Hemodilution	36 ± 6	37 ± 6	36 ± 4	37 ± 2	37 ± 5	35 ± 4	36 ± 4	37 ± 4
Arterial base excess (mM)								
Control	3.2 ± 2.1	3.0 ± 1.5	2.9 ± 1.4	2.6 ± 2.0	2.8 ± 1.7	2.5 ± 1.3	2.4 ± 1.1	2.3 ± 1.5
Hemodilution	3.7 ± 1.5	3.4 ± 1.8	3.1 ± 1.9	3.0 ± 2.2	3.3 ± 1.9	2.9 ± 2.2	2.8 ± 1.4	2.0 ± 1.8*

Values are mean ± SD. * $P = 0.05$; † $P = 0.01$ *versus* baseline.

Baseline values for laser Doppler flowmetry ranged from 12 to 96 perfusion units. The values were similar in both groups and in both control and flap tissues. Flap dissection resulted in a decrease in microcirculatory blood flow in the contralateral flap skin to 45 ± 18% of the preoperative value in the control group and to 53 ± 13% in the hemodilution group, whereas microcirculatory blood flow remained virtually unchanged in the other tissues. A transient, hemodilution-related increase was observed in the ipsilateral ($F = 0.81$, $P = 0.46$) and contralateral ($F = 2.75$, $P = 0.087$) flap skin (fig. 3).

At baseline, the mean tissue oxygen tension ranged from 37 ± 9 to 46 ± 7 mmHg in the control tissue and the ipsilateral flap tissue (fig. 4), whereas in the contralateral flap tissue, oxygen tension was reduced to 6.7 ± 4.3 (control group) and 7.6 ± 1.9 (hemodilution group) mmHg. Hemodilution had no significant effect on the tissue oxygen tension in the control and ipsilateral flap skin, but led to a transient increase in tissue oxygen tension in the contralateral flap ($F = 4.79$, $P = 0.019$), with a peak at a hemoglobin concentration of 9.1 g/dl. The measured value at this level of hemodilution was 17.0 ± 10.7 mmHg. Both the expected and the measured oxygen tension remained above baseline until a hemoglobin concentration of 6 g/dl was reached.

Flap dissection also resulted in a deterioration of base excess in the contralateral venous effluent to −5.6 ± 4.1 mM compared with 1.3 ± 1.6 mM to 2.7 ± 1.7 mM in the control and ipsilateral flap tissues (fig. 5). In both the control and the ipsilateral flap tissue, base excess de-

Table 2. Central Hemodynamics

Hemoglobin Concentration during Hemodilution (g/dl)	12	11	10	9	8	7	6	5
Cardiac output (l/min)								
Control	4.8 ± 1.5	4.6 ± 1.6	4.4 ± 1.4	4.6 ± 1.3	4.5 ± 1.4	4.6 ± 1.5	4.5 ± 1.4	4.6 ± 1.7
Hemodilution	4.5 ± 0.9	4.9 ± 1.3	5.6 ± 1.3	6.0 ± 2.0	6.0 ± 1.4*	6.4 ± 1.6†	6.8 ± 1.6†	6.9 ± 2.2†
Mean arterial pressure (mmHg)								
Control	107 ± 14	106 ± 15	106 ± 11	107 ± 18	108 ± 16	109 ± 18	108 ± 13	107 ± 15
Hemodilution	104 ± 7	105 ± 6	107 ± 10	105 ± 10	101 ± 11	101 ± 16	106 ± 14	99 ± 7
Heart rate (beats/min)								
Control	91 ± 21	87 ± 19	84 ± 20	88 ± 28	90 ± 23	87 ± 15	87 ± 22	91 ± 15
Hemodilution	94 ± 13	100 ± 21	104 ± 20	101 ± 18	110 ± 22	111 ± 18*	110 ± 20	117 ± 17†
Central venous pressure (mmHg)								
Control	7.2 ± 2.6	7.3 ± 2.4	7.5 ± 2.5	7.8 ± 2.4	7.8 ± 2.3	7.6 ± 2.4	7.5 ± 2.5	7.4 ± 2.4
Hemodilution	6.2 ± 2.5	6.7 ± 2.1	6.0 ± 1.7	7.3 ± 2.2	6.6 ± 1.8	6.2 ± 1.9	6.4 ± 1.6	7.4 ± 2.1
Pulmonary arterial pressure (mmHg)								
Control	21 ± 5	21 ± 5	21 ± 5	22 ± 4	21 ± 4	20 ± 5	21 ± 4	21 ± 4
Hemodilution	20 ± 5	20 ± 2	20 ± 4	22 ± 3	20 ± 4	20 ± 5	20 ± 4	20 ± 4
Pulmonary wedge pressure (mmHg)								
Control	8.9 ± 2.1	9.0 ± 1.7	9.0 ± 1.9	9.5 ± 1.4	9.5 ± 1.4	9.3 ± 2.1	9.6 ± 2.2	8.3 ± 2.1
Hemodilution	8.9 ± 1.8	9.1 ± 1.9	8.9 ± 2.1	10.4 ± 1.5	9.2 ± 2.0	9.2 ± 2.3	9.3 ± 2.3	9.5 ± 1.8

Values are mean ± SD. * $P = 0.05$, † $P = 0.01$ *versus* baseline.

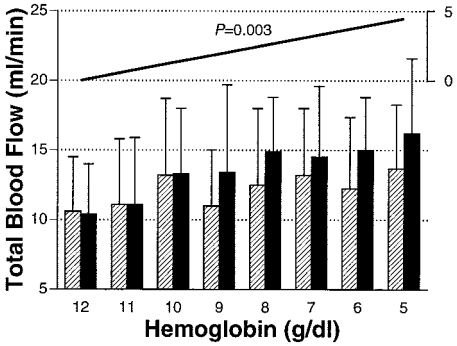

Fig. 2. Total blood flow to the flap measured by transit time flowmetry. The bar graph represents mean ± SD in the nondiluted control group (hatched bars) and during graded normovolemic hemodilution (solid bars). The line graph represents the expected change in total blood flow related to hemoglobin concentration. *P* is given for the linear regression of total blood flow on hemoglobin.

clined during hemodilution (F = 15.6 and $P = 0.001$ for control, $P = 0.016$ for ipsilateral flap). On the other hand, the base excess was transiently increased in the contralateral venous effluent because of hemodilution (F = 4.18, $P = 0.029$), reaching a maximum at a hemoglobin concentration of 8.5 g/dl and remaining above baseline until a hemoglobin concentration of 5 g/dl was reached.

Discussion

The pig has been regarded as an excellent experimental model for a variety of hemodynamic studies despite some physiologic properties differing from those collected during comparable conditions in humans.[16] However, the differences were mostly related to immaturity and vanished during growth, thus reaching values comparable to those in humans, which was the reason we chose adult minipigs for our experiments.[17] In the flap model used, arterial supply and venous drainage of the contralateral flap tissue were provided by a collateral vascular system linked to the anatomically perfused ipsilateral territory, which resulted in a marked reduction of microcirculatory blood flow and tissue oxygenation in the center of the contralateral part of the flap. Cell hypoxia and the subsequent metabolic acidosis in the ischemic tissue were reflected by the decreased base excess in the venous effluent. Therefore, our large animal model most closely simulates the clinical condition of a flap tissue undergoing ischemia and hypoxia. The anesthetic technique used in this study has been shown to provide stable hemodynamic conditions in flap sur-

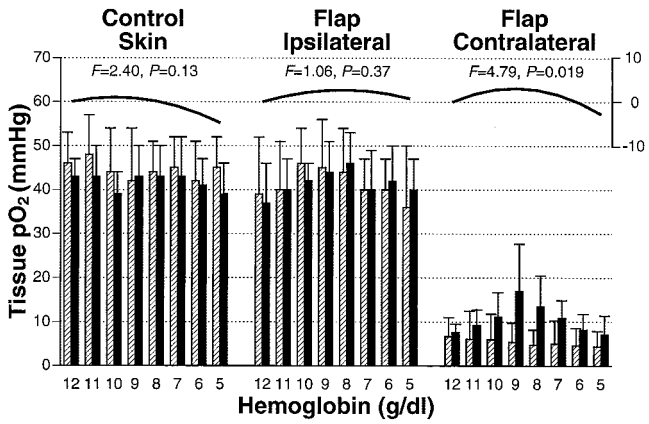

Fig. 4. Tissue oxygen tension measured by Clark probes in the control skin and the ipsilateral and contralateral flap skin. The bar graph represents mean ± SD in the nondiluted control group (hatched bars) and during graded normovolemic hemodilution (solid bars). The line graph represents the expected change in tissue oxygen tension related to hemoglobin concentration. F and *P* are given for the regression of tissue oxygen tension on hemoglobin squared and hemoglobin.

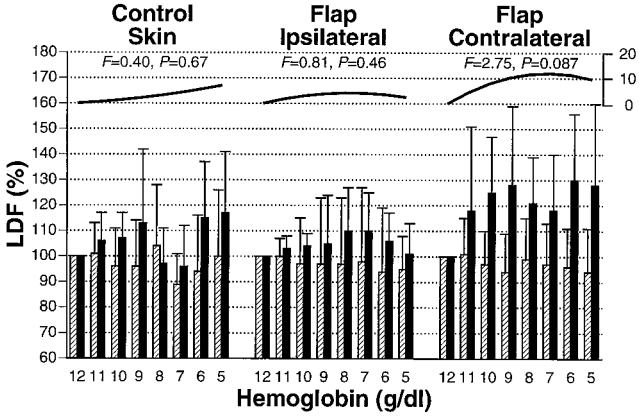

Fig. 3. Microcirculatory blood flow measured by laser Doppler flowmetry (LDF) in the control skin and the ipsilateral and contralateral flap skin. The bar graph represents mean ± SD in the nondiluted control group (hatched bars) and during graded normovolemic hemodilution (solid bars). The line graph represents the expected change in LDF related to hemoglobin concentration. F and *P* are given for the regression of LDF on hemoglobin squared and hemoglobin.

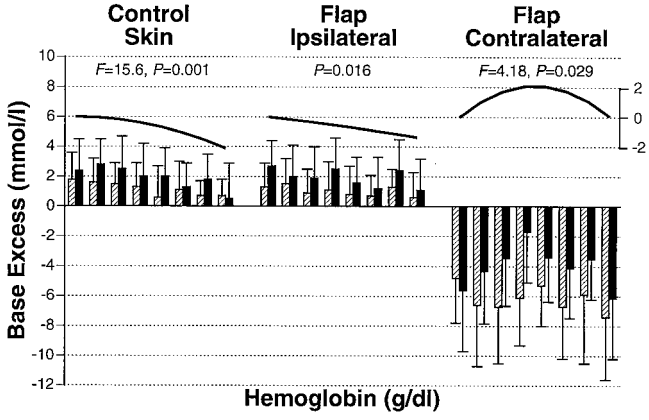

Fig. 5. Base excess in the venous effluent of the control skin and the ipsilateral and contralateral flap skin. The bar graph represents mean ± SD in the nondiluted control group (hatched bars) and during graded normovolemic hemodilution (solid bars). The line graph represents the expected change in base excess related to hemoglobin concentration. F and *P* are given for the regression of base excess on hemoglobin squared and hemoglobin.

gery.[12,13] In particular, we were able to show that there is no significant difference in microcirculatory blood flow in musculocutaneous flap tissue when halothane or isoflurane was used as long as no more than a 1.5 minimum alveolar halothane concentration is used and hypovolemia can be avoided.

The principal findings of the current study were that, in the ischemic and hypoxic flap tissue, both the oxygenation and metabolism were improved at a moderate degree of normovolemic hemodilution with hydroxyethyl starch and did not deteriorate even at a high level of hemodilution (hemoglobin concentration of 6 g/dl).

Normovolemic hemodilution is known to improve the microcirculatory blood flow in ischemic flap tissues at the expense of a reduction of oxygen carriers.[18,19] However, it may be assumed that the remaining oxygen carriers may not be capable of providing adequate oxygen supply to the ischemic tissue because of being exploited during the extended passage through the collateral arterial supply, since it has been shown that a large amount of oxygen may diffuse before reaching the capillaries in the normal skin.[10] On the other hand, we were able to demonstrate in an intravital microscopic study that the oxygen content was not reduced in the arteriolar inflow in the collateralized ischemic flap tissue after 50% hemodilution.[20]

In the current experiment, the loss of oxygen carriers may have been countered by the acceleration of blood flow, because it has been shown that the precapillary unloading of oxygen may be diminished by the reduced transit time of the erythrocytes as a result of hemodilution.[3] However, there were some discrepancies between the increments in total blood flow to the flap (measured with transit time flowmetry) and microcirculatory blood flow (measured with laser Doppler flowmetry). Similar discrepancies between total blood flow and microcirculatory blood flow in both muscle and skin have been observed in previous experiments on flap hemodynamics performed at our institution,[12,13] which makes it unlikely that the discrepancies are caused by a redistribution of flow from the skin to the muscle tissue, and which therefore suggests a methodologic origin. First, the laser Doppler signal reflects the product of hematocrit and mean blood cell velocity,[21] whereas transit time flowmetry is insensitive to the hematocrit.[14] The former would explain the parabolic behavior of the laser Doppler measurements obtained in the flap skin. Second, the flap tissue undergoes significant edema,[12] which may cause a reduction of the number of vascular structures assessed by the laser Doppler light and which may be increased during hemodilution. The aforementioned reasons indicate that the values for microcirculatory blood flow were underestimated in the flap tissue, in particular during hemodilution.

It is conceivable that the improvement of oxygenation in the contralateral flap tissue was achieved by the increased microcirculatory blood flow in this tissue. The increments for both the microcirculatory blood flow and the tissue oxygen tension were more significant in the contralateral flap tissue than ipsilaterally, indicating that the effect of hemodilution on the peripheral vascular resistance was more pronounced contralaterally. This may be because of an attenuation of erythrocyte aggregation[22] and leukocyte margination,[23] which are both promoted in low-flow conditions.

The maximal benefit of hemodilution on the oxygenation in the contralateral flap is expressed by the expected peak values for tissue oxygen tension and venous base excess, which occurred at hemoglobin concentrations of 9.1 and 8.5 g/dl, respectively (hematocrit, 27–29%). These values are slightly lower than those reported to provide maximal oxygenation in normal skin (hematocrit, 30–35%), which is determined by the product of erythrocyte velocity and microvascular hematocrit.[3,5] The shift of maximal oxygenation in the contralateral flap tissue to a lower level of systemic hemoglobin concentration and hematocrit may be explained by the hemoconcentration occurring in the flap tissue caused by fluid extravasation.[12]

In conclusion, we were able to demonstrate in a porcine model that normovolemic hemodilution may be a valid tool to improve the oxygenation in ischemic and hypoxic flap tissue. The best effect may be achieved at a hemoglobin concentration of 9 g/dl (hematocrit slightly $< 30\%$). Furthermore, our results suggest that flap ischemia *per se* does not represent an indication for the substitution of erythrocytes because of surgical or traumatic hemorrhage, since the transfusion trigger is commonly set at a level of hemoglobin concentration at which the oxygenation in the contralateral flap tissue was still improved.[2]

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