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Critical Hematocrit in Intestinal Tissue Oxygenation during Severe Normovolemic Hemodilution

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Background: **A critical point in oxygen supply for microvascular oxygenation during normovolemic hemodilution has not been identified. The relation between organ microvascular oxygen partial pressure (μPo_2) and organ oxygen consumption ($\dot{V}o_2$) during a decreasing oxygen delivery (Do_2) is not well understood. The present study was designed to determine the systemic hematocrit and organ Do_2 values below which organ μPo_2 and $\dot{V}o_2$ cannot be preserved by regulatory mechanisms during normovolemic hemodilution.**

Methods: **Eighteen male Wistar rats were randomized between an experimental group (n = 12), in which normovolemic hemodilution was performed with pasteurized protein solution (PPS), and a control group (n = 6). Systemic hemodynamic and intestinal oxygenation parameters were monitored. Intestinal μPo_2 was measured using the oxygen-dependent quenching of palladium-porphyrin phosphorescence.**

Results: **Baseline values in hemodilution and control group were similar. Hemodilution decreased hematocrit to 6.2 ± 0.8% (mean ± SD). Constant central venous pressure measurements suggested maintenance of isovolemia. Despite an increasing mesenteric blood flow, intestinal Do_2 decreased immediately. Initially, μPo_2 was preserved, whereas mesenteric venous Po_2 ($P_{mv}o_2$) decreased; below a hematocrit of 15%, μPo_2 decreased significantly below $P_{mv}o_2$. Critical Do_2 was 1.5 ± 0.5 ml · kg^{-1} · min^{-1} for $\dot{V}o_2$, and 1.6 ± 0.5 ml · kg^{-1} · min^{-1} for μPo_2. Critical hematocrit values for $\dot{V}o_2$ and μPo_2 were 15.8 ± 4.6% and 16.0 ± 3.5%, respectively.**

Conclusions: **Intestinal μPo_2 and $\dot{V}o_2$ were limited by a critical decrease in Do_2 and hematocrit at the same time. Beyond these critical points not only shunting of oxygen from the microcirculation could be demonstrated, but also a significant correlation between intestinal μPo_2 and $\dot{V}o_2$.**

NORMOVOLEMIC hemodilution can be used to delay or omit the need for transfusion of blood in a patient. Although the oxygen-carrying capacity of the blood is reduced during this procedure, the body oxygen consumption ($\dot{V}o_2$) is maintained by compensatory mechanisms such as an increase in cardiac output and an increase in the oxygen extraction ratio (O_2ER) of the tissues. However, there is a limit to this process: when the systemic oxygen delivery (Do_2) falls below a critical point, the compensatory mechanisms will be insufficient; $\dot{V}o_2$ becomes dependent on supply and will decrease at the same rate as the Do_2.[1] Such a critical point has been documented for systemic Do_2 and $\dot{V}o_2$ during normovolemic hemodilution in both anesthetized animals[2–6] and humans,[7,8] but does not provide information about the critical level of hemodilution for the different organ systems.

A critical point for the microvascular oxygenation during normovolemic hemodilution has not yet been determined. The relation between microvascular oxygen partial pressure (μPo_2) and tissue $\dot{V}o_2$ in this process is not well documented either. The present study was designed to determine the critical points at which the intestinal $\dot{V}o_2$ and μPo_2 could not be maintained by systemic or local compensatory mechanisms during extreme normovolemic hemodilution in the anesthetized rat. By comparing the regional venous Po_2 with the μPo_2, as measured with the oxygen-dependent quenching of palladium (Pd)-porphyrin phosphorescence, a direct measurement of the severity of the shunting of oxygen from the microcirculation can be obtained.[9] Our study focused on the microcirculation of the gut, as several investigations have shown that the intestinal $\dot{V}o_2$ is limited by supply at an earlier stage than systemic $\dot{V}o_2$.[2,10] Thus, this organ might be considered relatively sensitive to conditions of decreased systemic Do_2.[11]

Materials and Methods

Animals

The protocol of the present study was approved by the Animal Research Committee of the Academic Medical Center at the University of Amsterdam. Animal care and handling were performed in accordance with the national guidelines for care of laboratory animals. The experiments were performed in 18 male Wistar rats with a mean (± SD) body weight of 337 ± 23 g.

Preparation

The rats were anesthetized with an intraperitoneal injection of a mixture of 90 mg/kg ketamine, 0.5 mg/kg medetomidine, and 0.05 mg/kg atropine. Anesthesia was maintained with 50 mg · kg^{-1} · h^{-1} ketamine administered intravenously. To compensate for fluid loss, crystalloid solution was administered continuously at a rate of 15 ml · kg^{-1} · h^{-1}. Body temperature was measured with a thermocouple placed in the rectum and was maintained at 37 ± 0.5°C with a heating pad below and a warming lamp above the animal. Tracheotomy was performed, and a polyvinylchloride tube (charrière 6)

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was inserted into the trachea to enable mechanical ventilation with a mixture of 30% oxygen and 70% nitrogen. A heat and moisture exchanger (Humid-Vent Micro, Gibeck, Sweden) was placed between the tracheal tube and the ventilator to diminish loss of fluid through the mechanical ventilation. A capnometer (Capstar-100, CWE, Inc., Ardmore, PA) was used to measure end-tidal carbon dioxide partial pressure (P_{CO_2}). This information was used to adjust ventilator settings to maintain an arterial P_{CO_2} between 35 and 40 mmHg.

A polyethylene catheter (OD, 0.9 mm) was inserted into the right jugular vein for intravenous administration of drugs and fluids. The tip of the catheter was advanced near the right atrium for central venous blood sampling. A similar catheter was placed into the right carotid artery and connected to a pressure transducer for continuous monitoring of arterial blood pressure and heart rate. Catheters of the same size were placed into both the right femoral artery and vein for withdrawal of blood and administration of fluid. After midline laparotomy, a polyvinylchloride catheter (OD, 0.9 mm) was placed in the urinary bladder to prevent distension of the bladder wall and to monitor the urine production during the experiment.

The gut was exteriorized carefully and a 0.5-mm perivascular flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the superior mesenteric artery and connected to a flow meter (T206, Transonic Systems Inc.). For mesenteric venous blood sampling, an ileocecal vein was isolated under the microscope, ligated distally, and cannulated with a polyethylene catheter (OD, 0.8 mm). The tip of this catheter was advanced into the vessel in a proximal direction to have an estimate of the mesenteric venous blood gas values. A thermocouple was placed on the intestinal surface, and all exposed organ surfaces were covered with plastic foil to prevent evaporative fluid loss.

Measurements

Arterial pressure was measured in the carotid artery. Mean arterial pressure (MAP; mmHg) was calculated as MAP = diastolic pressure + (systolic pressure − diastolic pressure)/3. The amplitude of the arterial blood pressure was calculated as pulse pressure (P_{puls}) = systolic pressure − diastolic pressure. Blood flow in the superior mesenteric artery, $\dot{Q}_{sma}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) was measured continuously and indexed according to body weight. Urine output through the urinary bladder catheter was monitored. To avoid bias by differences in the time needed for preparation of the animal, the volume of produced urine at each measurement point was normalized to the urine volume at baseline 1. Blood samples of 0.2 ml each were collected and replaced with the same volume of pasteurized protein solution (PPS; CLB, Amsterdam, the Netherlands). At each measurement point, an arterial sample was taken from the femoral artery, a central venous sample was taken from the jugular venous catheter, and a mesenteric venous sample was taken from the ileocecal venous catheter. The samples were used to determine blood gas values (ABL505, Radiometer, Copenhagen, Denmark), as well as hematocrit, hemoglobin concentration, and hemoglobin oxygen saturation (OSM 3, Radiometer).

Intestinal D_{O_2} was calculated as $D_{O_{2,int}}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) = Q_{sma} · arterial oxygen content, which was calculated as (1.31 × [hemoglobin] × Sa_{O_2}) + (0.003 × Pa_{O_2}). Intestinal $\dot{V}_{O_2}$ was calculated as $\dot{V}_{O_{2,int}}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) = Q_{sma} × (arterial − mesenteric venous oxygen content difference). Mesenteric venous $\dot{V}_{O_2}$ was calculated as (1.31 × [hemoglobin] × $S_{mv}O_2$) + (0.003 × $P_{mv}O_2$). The intestinal oxygen extraction ratio was calculated as O_2ER_{int} (%) = $\dot{V}_{O_{2,int}}/D_{O_{2,int}}$. Because values of mesenteric or portal venous pressure were not available, an estimation of the vascular resistance of the superior mesenteric flow region was made: MAP:Q_{sma} ratio (U) = [MAP/Q_{sma}] × 100.

The intestinal μP_{O_2} was measured using the oxygen-dependent quenching of Pd-porphyrin phosphorescence.[12–14] Excitation of Pd-porphyrin by a pulse of light causes emission of phosphorescence with a decay in time, which is quantitatively related to the oxygen concentration. Pd-meso-tetra(4 carboxy-phenyl)porphine (Porphyrin Products, Logan, UT) is coupled to human serum albumin to form a large molecular complex that, when injected intravenously, is confined mainly to the vascular compartment.[13,15] One milliliter of a 4-mM Pd-porphyrin solution was administered, corresponding with a dosage of 12 mg/kg body weight. The μP_{O_2} measurements were made with an optical fiber for transmission of excitation and emission light, attached to a phosphorimeter. To determine which microvascular compartment is measured by fiber phosphorimetry, the Pd-porphyrin phosphorescence fiber technique has been compared with a microscopic phosphorimeter.[16] Simultaneous P_{O_2} measurements with the fiberoptic technique showed excellent correlation with microscopically measured P_{O_2} in capillaries and first-order venules, but not with arteriolar and venous P_{O_2} values, at different F_{IO_2} levels.[16] This study allowed us to term the fiberoptic measurement of P_{O_2} as the measurement of μP_{O_2}. The fiber was placed near the serosal surface of the terminal ileum. Fiberoptic measurements of μP_{O_2} incorporate blood vessels under the fiber over an area of approximately 1 cm^2 to a penetration depth of approximately 0.5 mm.[13,17] Because the calibration constants in the calculation of μP_{O_2} from the phosphorescence decay time are temperature-dependent, temperature measurements from the intestinal surface were used for correction of these constants.

Experimental Procedure

After surgery and stabilization, two baseline measurements were made during a 1-h period. At this point, the animals were randomized between the hemodilution (n = 12) and the control group (n = 6). Normovolemic hemodi-

lution was accomplished by withdrawal of blood from the femoral artery and simultaneous administration through the femoral vein of PPS at the same rate. The oncotic pressures of this protein solution and rat blood were determined, and it was found that the protein solution is slightly hyperoncotic compared with rat blood (oncotic pressures of 14.5 ± 0.4 and 12.7 ± 0.6 mmHg for PPS and rat blood, respectively) and therefore suitable for isovolemic hemodilution. Infusion–withdrawal occurred at a rate of 20 ml/h, using a double syringe pump (Harvard 33 syringe pump, Harvard Apparatus, South Natick, MA), and did not cause any undesired hemodynamic reactions in our model. Four dilution steps were made: from baseline down to a hematocrit of approximately 25% (H1), then to 15% (H2), to 10% (H3), and finally to 5–10% (H4). A 15-min stabilization period followed each dilution step before measurements were made. Because hematocrit values lower than 5% were found to be incompatible with life in this model, the experiments were terminated after measurement H4; an overdose of pentobarbital (60 mg intravenously) was administered to the animals. In the control group, no hemodilution was performed, but measurements were made at similar time intervals as in the hemodilution group.

To provide more information on the effect of the hemodilution procedure on the animals' volume status, in four additional animals the arterial and central venous pressures were measured during hemodilution in combination with measurement of the intestinal μPo_2. Hemodilution was performed in the same way as in the other animals, and subsequently a fluid challenge of 2.5 ml PPS was administered, which could be expected to cause an increase in MAP if the animal would be hypovolemic.

Statistical Analysis

Values are reported as mean ± SD. Data within each group were analyzed using analysis of variance for repeated measurements. When appropriate, *post hoc* analyses were performed with the Student-Newman-Keuls test. $P_{mv}O_2$ and μPo_2 were compared at each measurement point with the Student paired *t* test. The hemodilution and the control group were compared with an unpaired *t* test. *P* values less than 0.05 were considered significant. The critical level of hemodilution was determined from plots of hematocrit and $Do_{2,int}$ against both μPo_2 and $\dot{V}o_{2,int}$. The critical points were defined as the points at which μPo_2 and $\dot{V}o_{2,int}$ became dependent on hematocrit and $Do_{2,int}$ with further hemodilution. These points were determined for each animal separately by the intersection of the two best-fit regression lines with a least sum of squares technique.[1] The correlation between μPo_2 and $\dot{V}o_{2,int}$ was calculated with the Pearson correlation coefficient.

Results

Systemic Hemodynamics

On completion of the experiment, an average of 26.3 ± 5.1 ml blood had been exchanged for an identical volume of PPS. Baseline values in the hemodilution and the control group were not different. As shown in table 1, hemodilution decreased hematocrit from 44.9 ± 3.8% at baseline to 6.2 ± 0.8% at H4 and [hemoglobin] from 14.6 ± 1.3 g/dl to 1.8 ± 0.3 g/dl. Although hematocrit and [hemoglobin] decreased in the control group as well, to 35.1 ± 1.3% and 11.6 ± 0.5 g/dl at H4, respectively, the values in the hemodilution group were significantly lower from control from H1 to H4. MAP, $\dot{Q}_{sma}$, heart rate, and MAP:$\dot{Q}_{sma}$ ratio did not change in the control group. In the experimental group, MAP (106 ± 13 mmHg at baseline) decreased significantly at H2 (corresponding hematocrit, 14.8 ± 2.8%) to 85 ± 23 mmHg and reached a minimum of 46 ± 11 mmHg at H4. Heart rate (267 ± 20 beats/min at baseline) decreased significantly to 241 ± 35 beats/min at H3 and to 231 ± 35 beats/min at H4. During hemodilution, P_{puls} increased significantly compared with the control group, from 13 ± 5 mmHg at baseline 1 to 30 ± 13 mmHg at H3. P_{puls} did not change significantly in the control group. $\dot{Q}_{sma}$ (14.0 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$ at baseline) increased at H1 to 17.5 ± 2.0 $ml \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$), reached a maximum value of 21.9 ± 3.3 $ml \cdot kg^{-1} \cdot min^{-1}$ at H3, and returned to baseline values at H4. The MAP:$\dot{Q}_{sma}$ ratio (2403 ± 466 U at baseline) decreased progressively to 861 ± 324 U at H4. Again, significance was reached at H1 (1810 ± 431 U). The urine production was normalized to the amount at the start of the experiment and was similar in the hemodilution and the control group. A summary of these data is given in table 1.

Oxygen Measurements

Results of the oxygen measurements and related parameters are shown in tables 2–4. In the hemodilution group, mesenteric venous Po_2 ($P_{mv}O_2$) (60 ± 8 mmHg at baseline) decreased significantly at H1 (42 ± 4 mmHg) to 34 ± 4 mmHg at H4. Intestinal μPo_2 in the same group, however, remained stable around 60 mmHg until H2, then it decreased to 25 ± 3 mmHg at H3 to a final value of 14 ± 4 mmHg at H4. Comparing μPo_2 to $P_{mv}O_2$ (fig. 1), it is shown that the $P_{mv}O_2$ became significantly lower than the μPo_2 at H1 ($P < 0.05$). However, in contrast to this, at H3 and H4 (when hematocrit was decreased below 10%), μPo_2 had fallen below $P_{mv}O_2$ ($P < 0.05$). The figure clearly shows the difference with the control group, in which both parameters did not change.

Mesenteric venous oxygen saturation ($S_{mv}O_2$) was 77 ± 9% at baseline, started to decrease at H1 to 67 ± 8% ($P < 0.05$), with a final value of 33 ± 8% at H4. A similar pattern was observed for the central venous oxygen saturation ($S_{cv}O_2$), with values of 61 ± 12 at baseline to 45 ± 13 at H1 ($P < 0.05$) and 24 ± 7 at H4. In the control group, $S_{mv}O_2$

Table 1. Systemic Hemodynamics during Normovolemic Hemodilution

	Baseline 1	Baseline 2	H1	H2	H3	H4
Ht (%)						
H	44.9 ± 3.8	43.2 ± 3.4	24.6 ± 4.8*†	14.8 ± 2.8*†‡	9.0 ± 1.5*†‡	6.2 ± 0.8*†‡
C	43.3 ± 0.8	42.2 ± 1.5	39.5 ± 2.0*	38.5 ± 2.2*	37.6 ± 1.6*	35.1 ± 1.3*‡
[Hb] (g/dl)						
H	14.6 ± 1.3	13.9 ± 1.2	7.9 ± 1.6*†	4.6 ± 0.9*†‡	2.7 ± 0.5*†‡	1.8 ± 0.3*†‡
C	13.9 ± 0.5	13.6 ± 0.6	13.0 ± 0.8	12.3 ± 0.7*	12.4 ± 0.3*	11.6 ± 0.5*
HR (beats/min)						
H	267 ± 20	267 ± 20	255 ± 18	248 ± 35	242 ± 35*	231 ± 35*
C	258 ± 30	265 ± 22	258 ± 16	258 ± 24	263 ± 27	253 ± 16
MAP (mmHg)						
H	106 ± 13	105 ± 14	94 ± 20	85 ± 23*‡	60 ± 18*†‡	46 ± 11*†‡
C	107 ± 3	109 ± 4	107 ± 7	105 ± 6	104 ± 5	104 ± 5
P_{puls} (mmHg)						
H	13 ± 5	14 ± 5	20 ± 7*†	23 ± 7*†	30 ± 7*†‡	28 ± 10*†
C	10 ± 2	10 ± 4	9 ± 2	10 ± 3	14 ± 5	12 ± 4
Q_{sma} (ml · kg^{-1} · min^{-1})						
H	14.0 ± 2.3	13.6 ± 2.0	17.5 ± 2.0*†	19.2 ± 3.2*†	21.9 ± 3.3*†‡	12.3 ± 4.4‡
C	14.5 ± 1.6	13.8 ± 1.8	13.4 ± 1.8	13.7 ± 1.8	13.6 ± 1.2	13.5 ± 1.8
MAP/Q_{sma} ratio (U)						
H	2,403 ± 466	2,422 ± 430	1,810 ± 431*†	1,437 ± 438*†‡	887 ± 324*†‡	861 ± 324*†
C	2,368 ± 283	2,547 ± 359	2,476 ± 250	2,388 ± 181	2,389 ± 169	2,410 ± 211
Urine production (normalized)						
H	1.0	1.2 ± 0.5	1.4 ± 0.6	1.6 ± 0.6	1.7 ± 0.5	1.7 ± 0.7
C	1.0	1.2 ± 0.2	1.3 ± 0.3	1.5 ± 0.4	1.6 ± 0.5	1.7 ± 0.5

Values represent mean ± SD.

* $P < 0.05$ *versus* baseline (1 and 2). † $P < 0.05$ *versus* control. ‡ $P < 0.05$ *versus* previous.

H = hemodilution group; C = control group; Ht = hematocrit; [Hb] = hemoglobin concentration; HR = heart rate; MAP = mean arterial blood pressure; P_{puls} = pulse pressure; Q_{sma} = superior mesenteric artery blood flow.

did not change, and $S_{cv}O_2$ decreased significantly at H3 and H4.

Despite an increased $\dot{Q}_{sma}$, $Do_{2,int}$ decreased immediately when hematocrit was diminished by hemodilution, from 2.6 ± 0.3 ml · kg^{-1} · min^{-1} at baseline to 0.4 ± 0.1 ml · kg^{-1} · min^{-1} at H4. Initially, $\dot{V}o_{2,int}$ remained constant at 0.6 ml · kg^{-1} · min^{-1} but decreased significantly at H3 (0.5 ± 0.1 ml · kg^{-1} · min^{-1}) and reached 0.3 ± 0.1 ml · kg^{-1} · min^{-1} at H4. At H1, the O_2ER_{int} was significantly increased compared with baseline (from 25 ± 8% at baseline to 34 ± 8% at H1) and continued to increase to 70 ± 6% at H4. In the control group, $\dot{V}o_{2,int}$ remained constant around 0.6 ml · kg^{-1} · min^{-1} throughout the experiment. However, $Do_{2,int}$ (2.6 ± 0.2 ml · kg^{-1} · min^{-1} at baseline) decreased slightly but significantly to 2.1 ± 0.1 at H4, which was still significantly higher than in the hemodilution group. As a result, the O_2ER_{int} in the control group (20 ± 2% at baseline) was increased at H2 ($P < 0.05$) to 29 ± 3% at H4.

Arterial *p*H and Sa_{O_2} did not change in either group. Pa_{O_2} did not change in the control group (171 ± 18 mmHg at baseline) but increased during hemodilution from 165 ± 17 mmHg at baseline to 194 ± 15 mmHg at H4. Arterial Pc_{O_2} in the control group remained constant around

Table 2. Intestinal Oxygenation Parameters

	Baseline 1	Baseline 2	H1	H2	H3	H4
Do_2 (ml · kg^{-1} · min^{-1})						
H	2.6 ± 0.3	2.5 ± 0.5	1.9 ± 0.5*	1.3 ± 0.3*†‡	0.9 ± 0.2*†‡	0.4 ± 0.1*†‡
C	2.6 ± 0.2	2.5 ± 0.2	2.3 ± 0.2*	2.2 ± 0.2*	2.2 ± 0.2*	2.1 ± 0.1*
$\dot{V}o_2$ (ml · kg^{-1} · min^{-1})						
H	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1†‡	0.3 ± 0.1*†‡
C	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.1
O_2ER (%)						
H	25 ± 8	27 ± 11	34 ± 8†‡	44 ± 5*†‡	56 ± 8*†‡	70 ± 6*†‡
C	20 ± 2	21 ± 6	22 ± 2	27 ± 6*	27 ± 2*	29 ± 3*

Values represent mean ± SD.

* $P < 0.05$ *versus* baseline (1 and 2). † $P < 0.05$ *versus* previous. ‡ $P < 0.05$ *versus* control.

H = hemodilution group; C = control group; Do_2 = mesenteric oxygen delivery; $\dot{V}o_2$ = mesenteric oxygen consumption; O_2ER = mesenteric oxygen extraction ratio.

Table 3. Blood Gas Measurements

	Baseline 1	Baseline 2	H1	H2	H3	H4
Pa_{CO_2} (mmHg)						
H	38 ± 4	36 ± 3	37 ± 4	34 ± 3	31 ± 6*	23 ± 7*†‡
C	37 ± 7	37 ± 5	35 ± 4	36 ± 4	36 ± 5	37 ± 5
Sa_{O_2} (%)						
H	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1
C	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 1
pH_a						
H	7.35 ± 0.05	7.35 ± 0.06	7.36 ± 0.05	7.36 ± 0.05	7.36 ± 0.05	7.38 ± 0.05
C	7.35 ± 0.06	7.36 ± 0.05	7.36 ± 0.04	7.36 ± 0.04	7.36 ± 0.02	7.36 ± 0.02
$S_{mv}O_2$ (%)						
H	77 ± 9	72 ± 12	67 ± 8‡	57 ± 5*†‡	49 ± 9*†‡	33 ± 8*†‡
C	77 ± 4	79 ± 6	76 ± 6	77 ± 5	76 ± 4	73 ± 4
pH_{mv}						
H	7.33 ± 0.04	7.32 ± 0.05	7.33 ± 0.08	7.32 ± 0.07	7.26 ± 0.07*†‡	7.19 ± 0.06*†‡
C	7.35 ± 0.02	7.34 ± 0.05	7.33 ± 0.03	7.34 ± 0.03	7.33 ± 0.04	7.34 ± 0.05
$P_{mv}CO_2$ (mmHg)						
H	46 ± 5	47 ± 5	47 ± 5	46 ± 4	47 ± 5	49 ± 8
C	43 ± 4	40 ± 5	44 ± 5	43 ± 3	44 ± 2	43 ± 2
$S_{cv}O_2$ (%)						
H	61 ± 12	54 ± 13	45 ± 13*‡	31 ± 8*†‡	29 ± 10*‡	24 ± 7*‡
C	62 ± 6	64 ± 4	62 ± 5	57 ± 10	54 ± 7*	51 ± 11*

Values represent mean ± SD.

* $P < 0.05$ *versus* baseline (1 and 2). † $P < 0.05$ *versus* previous. ‡ $P < 0.05$ *versus* control.

H = hemodilution group; C = control group; Pa_{CO_2} = arterial carbon dioxide partial pressure; Sa_{O_2} = arterial oxygen saturation; pH_a = arterial *p*H; $S_{mv}O_2$ = mesenteric venous oxygen saturation; pH_{mv} = mesenteric venous *p*H; $P_{mv}CO_2$ = mesenteric venous carbon dioxide tension; $S_{cv}O_2$ = central venous oxygen saturation.

37 mmHg; in the hemodilution group (38 ± 4 mmHg at baseline) P_{CO_2} decreased at H3 to 23 ± 7 mmHg at H4.

In the control group, mesenteric venous *p*H and P_{CO_2} were stable around 7.34 and 43 mmHg, respectively. After hemodilution, $P_{mv}CO_2$ did not change from baseline or control. However, pH_{mv} decreased significantly at H3 (7.26 ± 0.07) and H4 (7.19 ± 0.06).

The critical $D_{O_{2,int}}$ and hematocrit values are shown in figures 2 and 3. $\dot{V}_{O_{2,int}}$ became dependent on supply at a $D_{O_{2,int}}$ of 1.5 ± 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$. For the μP_{O_2}, critical $D_{O_{2,int}}$ was 1.6 ± 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$. A similar critical value was calculated for the hematocrit: $\dot{V}_{O_{2,int}}$ decreased with hematocrit at 15.8 ± 4.6%. The critical hematocrit for μP_{O_2} was 16.0 ± 3.5%. The critical D_{O_2} values and hematocrit values were not significantly different for μP_{O_2} and $\dot{V}_{O_{2,int}}$, indicating that $\dot{V}_{O_{2,int}}$ and μP_{O_2} became dependent on supply at the same point during hemodilution. This is illustrated in figure 4: during intact oxygenation, little correlation could be found between μP_{O_2} and $\dot{V}_{O_{2,int}}$. As soon as intestinal oxygenation was impaired by progressive hemodilution, a significant correlation between intestinal μP_{O_2} and $\dot{V}_{O_{2,int}}$ could be demonstrated (Pearson $r^2 = 0.86$; $P < 0.0001$).

Central Venous Pressure Measurements

The results of the combined measurement of the central venous pressure, MAP, and intestinal μP_{O_2} during hemodilution in four animals are shown in figure 5. The central venous pressure increased slightly but not significantly during hemodilution. The MAP and the intestinal

Table 4. P_{O_2} Measurements

	Baseline 1	Baseline 2	H1	H2	H3	H4
Pa_{O_2} (mmHg)						
H	165 ± 17	165 ± 15	171 ± 15	183 ± 19*†	190 ± 15*	194 ± 15*
C	171 ± 18	182 ± 15	180 ± 18	184 ± 17	183 ± 17	181 ± 15
$P_{mv}O_2$ (mmHg)						
H	60 ± 8	56 ± 10	53 ± 8	42 ± 4*†‡	37 ± 7*‡	34 ± 4*‡
C	60 ± 5	62 ± 6	59 ± 6	58 ± 5	62 ± 3	57 ± 2
μP_{O_2} (mmHg)						
H	65 ± 4	63 ± 4	60 ± 3	54 ± 6	25 ± 3*†‡	14 ± 4*†‡
C	62 ± 6	62 ± 6	61 ± 5	60 ± 4	58 ± 3	60 ± 5

Values represent mean ± SD.

* $P < 0.05$ *versus* baseline (1 and 2). † $P < 0.05$ *versus* previous. ‡ $P < 0.05$ *versus* control.

P_{O_2} = partial pressure of oxygen; H = hemodilution group; C = control group; Pa_{O_2} = arterial P_{O_2}; $P_{mv}O_2$ = mesenteric venous P_{O_2}; μP_{O_2} = intestinal microvascular P_{O_2}.

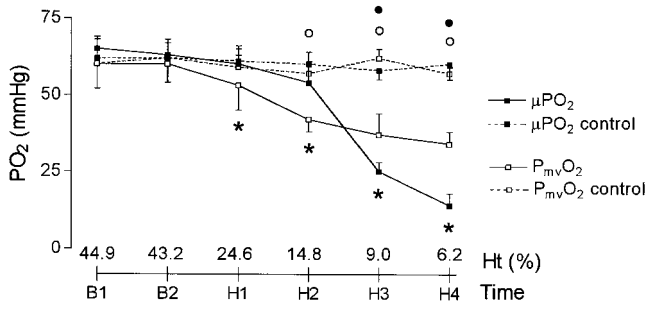

Fig. 1. Mesenteric venous oxygen partial pressure ($P_{mv}O_2$) and intestinal microvascular oxygen partial pressure (μPo_2). ●μPo_2: $P < 0.05$ *versus* baseline; ○$P_{mv}O_2$: $P < 0.05$ *versus* baseline; *$P_{mv}O_2$: $P < 0.05$ *versus* μPo_2. Until a hematocrit of 15% was reached, μPo_2 was preserved, resulting in a significant divergence between μPo_2 and $P_{mv}O_2$ at H1 and H2. In contrast, a Po_2 gap between venous and microvascular blood could be demonstrated with more severe hemodilution, indicating that at this stage, oxygen was being shunted from the microcirculation. In the control group, no change in μPo_2 or $P_{mv}O_2$ was observed. Ht = hematocrit.

μPo_2 showed a similar response to hemodilution as in the other animals. After the fluid challenge, hematocrit decreased from 8% to 6%, but the central venous pressure, MAP, and μPo_2 did not change significantly.

Discussion

The main result of this study is that a critical point in the intestinal microvascular oxygenation could be identified during extreme normovolemic hemodilution. The critical point for μPo_2 corresponded with the critical point for $\dot{V}o_{2,int}$: both $\dot{V}o_{2,int}$ and μPo_2 were limited by supply at the same stage during severe normovolemic hemodilution. Not only did we find similar critical $Do_{2,int}$ values for μPo_2 and $\dot{V}o_{2,int}$, but critical hematocrit values were of the same order as well. When the critical point in intestinal Do_2 had been reached and μPo_2 and $\dot{V}o_{2,int}$ were limited by supply, a significant correlation between the $\dot{V}o_{2,int}$ and the μPo_2 could be demonstrated, but not between $\dot{V}o_{2,int}$ and $P_{mv}o_2$. At this time, O_2ER_{int} had not reached a maximum yet, but continued to increase until the end of the experiment, indicating that regulation of the intestinal tissue oxygenation was still functioning, although insufficiently.

Until a hematocrit of ± 15% at H2, μPo_2 remained constant, resulting in a significant divergence between $\mu Po2$ and $P_{mv}o_2$ (fig. 1). This might be explained by the notion from other investigators that hemodilution can increase microvascular perfusion by increasing the amount of perfused capillaries (capillary recruitment) or by vasodilatation of microvessels already perfused.[18–20] As a result, the absolute amount of oxygen transported to the capillaries can be maintained and a more homogeneous distribution of the flow in the microcirculation is provided as well.[21,22] This shortens the oxygen diffusion distances and facilitates the oxygen uptake (increased extracting bed). In the regulation of the intestinal tissue oxygenation, capillary recruitment is of great importance.[23] In fact, it has been shown to be of greater quantitative significance than blood flow autoregulation in preventing cellular hypoxia when intestinal perfusion pressure is reduced.[24] In addition, changes that have been observed in skeletal muscle microcirculation after the decrease in blood viscosity during hemodilution can be assumed to contribute to the preservation of the intestinal tissue oxygenation as well: a decrease in the precapillary diffusional oxygen loss, resulting in more oxygen being delivered to the capillaries and a diminished oxygen diffusion path,[25–29] allowing a more efficient utilization of the remaining circulating erythrocyte volume.[30,31]

With more progressive hemodilution, $Do_{2,int}$ and hematocrit reached the critical point where μPo_2 became limited by the decreasing oxygen supply and decreased progressively below $P_{mv}o_2$. This resulted in a Po_2 gap between mesenteric venous and microvascular blood,

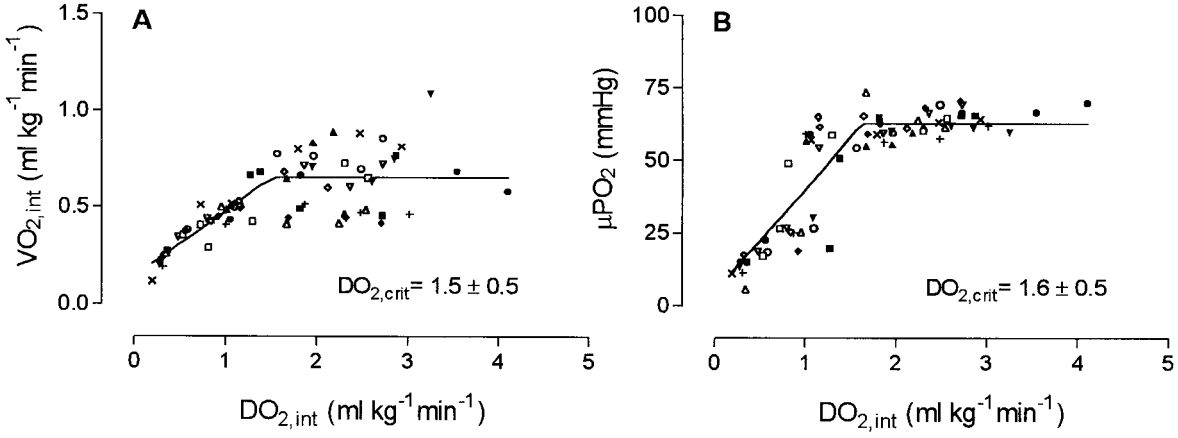

Fig. 2. (*A*) Critical oxygen delivery value (Do_2) for the intestinal oxygen consumption ($\dot{V}o_{2,int}$). (*B*) Critical Do_2 value for the intestinal microvascular oxygen partial pressure (μPo_2). Critical values were determined in each animal separately and are represented here as mean ± SD. Data originating from the same animal are represented by a similar symbol.

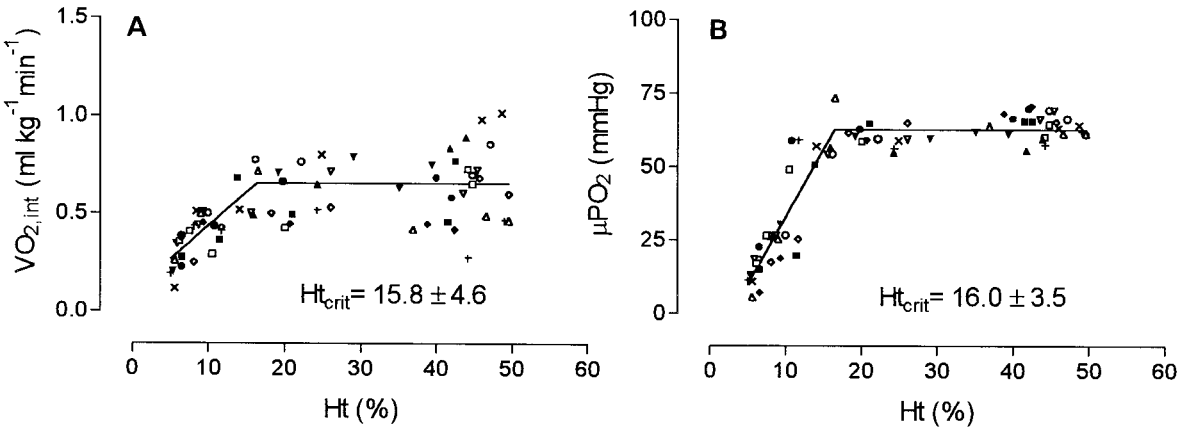

Fig. 3. (*A*) Critical hematocrit value (Ht_{crit}) for the intestinal oxygen consumption ($\dot{V}o_{2,int}$). (*B*)Critical hematocrit value for the intestinal microvascular oxygen partial pressure (μPo_2). Critical values were determined in each animal separately and are represented here as mean ± SD. Data originating from the same animal are represented by a similar symbol.

demonstrating that at this stage, oxygen was being shunted from the intestinal microcirculation. Principal mechanisms that can cause functional shunting within the microcirculation are: convective shunting through anatomical anastomoses, direct diffusion of oxygen from arterioles to venules, altered heterogeneity of the microvascular architecture leading to "vascular steal," and inability of hemoglobin to off-load oxygen fast enough to the tissues as it passes through the microcirculation.[9] Which of these mechanisms can be held responsible for the Po_2 gap during hemodilution has yet to be determined. Whether it is the origin or merely an effect of the inability of intestinal regulatory mechanisms to maintain tissue oxygenation is not clear.

The decrease in both $S_{mv}O_2$ and $S_{cv}O_2$ reflects the increase in O_2ER during the experiment. O_2ER_{int} was increased to 70% at H4, indicating that regulation of the intestinal oxygenation was capable of increasing O_2ER_{int}, although not sufficient to maintain the intestinal oxygenation at baseline levels. The similar critical values and the significant correlation between μPo_2 and $\dot{V}o_{2,int}$ in the state of oxygen supply dependency support the hypothesis that the amount of physically dissolved oxygen within the microcirculation can be considered as the major driving pressure for diffusion of oxygen into the tissue cells, and as such, can be regarded as an important determinant of tissue $\dot{V}o_2$.

Q_{sma} is an important determinant of the $Do_{2,int}$ and as such influences the critical hematocrit and $Do_{2,int}$ values during hemodilution. During hemodilution, Q_{sma} has been reported to increase sufficiently to maintain oxygen flux to the organs, including the small intestine, until a hematocrit of ± 10% is reached.[32] Although Q_{sma} increased significantly in this study, $Do_{2,int}$ decreased; the increase in Q_{sma} was never sufficient to fully compensate the decreased oxygen-carrying capacity of the blood. This observation has been reported in prior studies as well[2,33] and indicates that an increase in O_2ER is more important in the preservation of intestinal $\dot{V}o_2$.

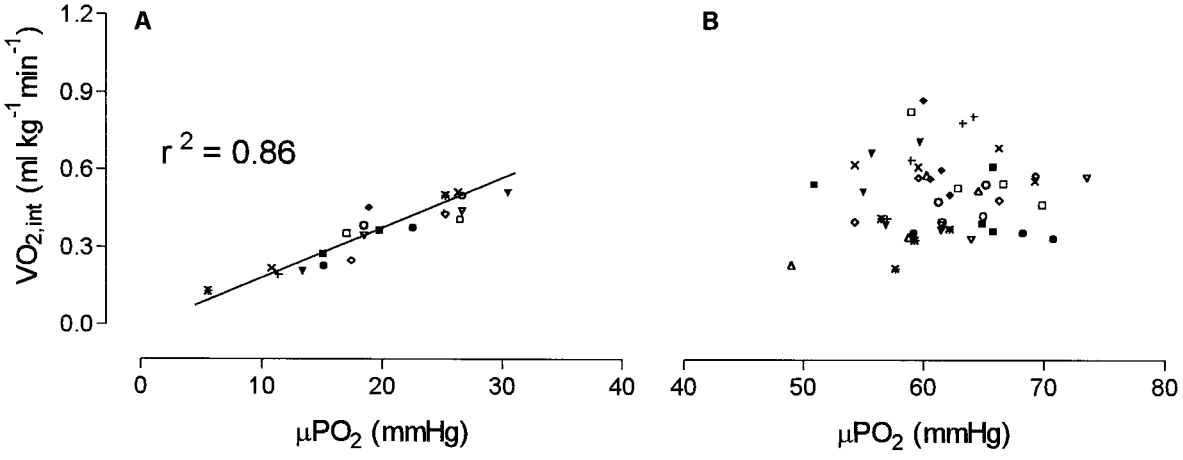

Fig. 4. Relation between intestinal microvascular oxygen partial pressure (μPo_2) and intestinal oxygen consumption ($\dot{V}o_{2,int}$). (*A*) During a state of oxygen supply dependency, a significant correlation between μPo_2 and intestinal $\dot{V}o_2$ could be demonstrated (Pearson $r^2 = 0.86$; $P < 0.0001$). (*B*) During uncompromised intestinal tissue oxygenation, there was little correlation between μPo_2 and intestinal $\dot{V}o_2$. Data originating from the same animal are represented by a similar symbol.

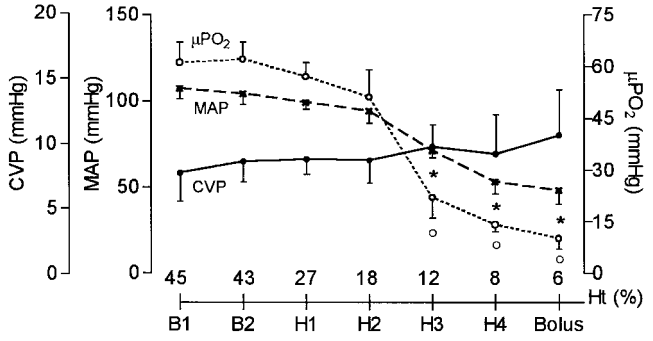

Fig. 5. Central venous pressure (CVP), mean arterial pressure (MAP), and intestinal microvascular oxygen partial pressure (μPo_2) during extreme normovolemic hemodilution in the rat. Stepwise hemodilution was performed with pasteurized protein solution. After hemodilution, a bolus dose of 2.5 ml pasteurized protein solution was infused over 10 min. Data represent mean ± SD (n = 4). ○ μPo_2: $P < 0.05$ *versus* baseline; *MAP: $P < 0.05$ *versus* baseline. MAP and μPo_2 decreased significantly, whereas CVP did not change throughout the experiment, indicating that isovolemia was maintained. Fluid challenge decreased hematocrit (Ht) from 8% to 6% but did not result in a significant change in the other parameters.

Despite an increase after hemodilution, Q_{sma} fell back to baseline values in the final phase of the experiment, when hematocrit was only 6%. Simultaneously, MAP decreased to 46 mmHg, and the heart rate was significantly decreased as well. Most likely, hemodynamic stability could no longer be maintained at this point. This is supported by the decrease in arterial Pco_2 at H4. It can be hypothesized that the substantial hypotension and decrease in Q_{sma} at the lowest hematocrit were caused by inadequate cardiac function.

The arterial *p*H did not change during the experiment, unlike the mesenteric venous *p*H; the latter decreased significantly at H3. This was the same point where both the intestinal μPo_2 and $\dot{V}o_{2,int}$ decreased significantly from baseline values, which not only supports the notion that now the intestinal tissue oxygenation had become impaired but also indicates that in this study, pH_{mv} was a more reliable indicator of intestinal tissue oxygenation than $P_{mv}o_2$.

One might argue that failure to maintain isovolemia throughout the experiment might explain these observations as well. In the absence of blood volume measurements, only supporting evidence could be provided regarding the maintenance of isovolemia during hemodilution, *i.e.*, the constant central venous pressure during hemodilution and the lack of effect of the subsequent fluid challenge on central venous pressure, MAP, and μPo_2 in four additional animals. In addition, a significant increase in P_{puls} in the hemodilution group, the comparable urine production in the hemodilution and the control groups, the use of a slightly hyperoncotic protein solution, and a slight increase in total body weight of the animals during the experiment suggest that the animals in the hemodilution group were not hypovolemic throughout the experiment. Therefore, the hemodynamic parameters and the determination of a critical level of Do_2 for the intestinal $\dot{V}o_2$ and μPo_2 in this model were not likely to be biased by an insufficient circulating volume.

Because of blood sampling and subsequent volume correction, a small but significant degree of hemodilution took place in the control group, resulting in a decrease in $Do_{2,int}$. $\dot{V}o_{2,int}$ and μPo_2 were preserved by an increase in O_2ER from 20% at baseline to 29% at H4. This was only reflected in the decrease in $S_{cv}o_2$; any change in the other parameters did not reach significance. The results from the control group demonstrated that the hemodynamic parameters were stable throughout the entire experiment.

In contrast with our results are the observations of Haisjackl *et al.*,[34] who observed no significant change in pig $\dot{V}o_{2,int}$ until a systemic hematocrit of ± 6% was reached, as a result of a doubling in intestinal O_2ER. This suggested an uncompromised microcirculatory oxygenation at an extremely low hematocrit. However, surface oxygen electrode measurements in their study demonstrated that mucosal Po_2 was preserved until a hematocrit of 6% was reached and that serosal Po_2 decreased below a hematocrit of 15%. The measurement depth of the Pd-porphyrin phosphorescence is in the order of 0.5 mm.[17] Therefore, the size of the rat small intestine did not allow us to distinguish between a mucosal and a serosal μPo_2, as would be possible when using Pd-porphyrin phosphorescence in the pig.[17,35] Thus, the intestinal μPo_2 values in our study, although in the same range as pig serosal Po_2 values measured with either oxygen electrode or Pd-porphyrin phosphorescence, must be considered as a resultant of the μPo_2 values in the different layers of the gut wall. As such, they are hardly comparable to μPo_2 values found in the pig. Furthermore, it has been shown that oxygen electrodes are sensitive to changes in arterial oxygen content.[9,17] The use of the oxygen-dependent quenching of Pd-porphyrin phosphorescence might be more advantageous as this technique has a deeper penetration depth and, used in combination with an optical fiber, selectively measures the Po_2 in the capillaries and the venules.[16,17] The discrepancy in intestinal $\dot{V}o_2$ might be explained by the interspecies difference (pig *vs.* rat), or by the fact that Haisjackl *et al.*[34] used an isolated gut segment, which was perfused at a constant pressure, whereas in our experiments the intestines were not isolated and were perfused at the MAP, which decreased significantly below hematocrit 15%. This is supported by the results from Nöldge *et al.*,[36] who found a decrease in both MAP and liver and small intestine surface Po_2 values at and below a hematocrit of 15% during normovolemic hemodilution. In this context, it must be realized that for an adequate interpretation of the results of hemodilution experiments, the use of anesthesia must be taken into account: the compensatory mechanisms during hemodi-

lution are influenced by the use of anesthesia and a critical level of Do_2 could not be demonstrated during hemodilution in conscious dogs and humans.[37–39]

In conclusion, a critical point for the intestinal tissue oxygenation could be identified during normovolemic hemodilution. It was demonstrated that μPo_2 and $\dot{V}o_{2,int}$ were limited by oxygen supply at the same time when the intestinal Do_2 decreased to a critical level. As soon as the intestinal oxygenation became dependent on oxygen supply, pH_{mv} decreased and an increasing Po_2 gap between the venous and microvascular blood could be demonstrated, reflecting shunting of oxygen from the microcirculation. At this point, a significant correlation between μPo_2 and $\dot{V}o_{2,int}$ could be demonstrated, but not between $P_{mv}o_2$ and $\dot{V}o_{2,int}$. These data indicate that as soon as the μPo_2 is impaired during hemodilution, the tissue $\dot{V}o_2$ is affected as well. Unlike the regional venous *p*H, venous Po_2 cannot be regarded as a reliable parameter for the judgment of the intestinal tissue oxygenation.

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