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Effects of Acute Normovolemic Hemodilution on Splanchnic Oxygenation and on Hepatic Histology and Metabolism in Anesthetized Pigs

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Perioperative hemodilution (HD) has become an accepted means of reducing transfusion requirements. Therefore, the effects of limited (decrease in hematocrit [Hct] from 30 to 20%, "HD1") and severe (decrease in Hct from 20 to 14%, "HD2") acute normovolemic HD with 6% hydroxyethyl starch on splanchnic blood flows (electromagnetic flow probes), O_2 uptakes and deliveries, surface O_2 tensions (P_{O_2}) (Clark-type electrode), hepatic metabolism (organic acids), and hepatic histology (liver biopsies) were studied in nine pigs anesthetized and paralyzed with ketamine/flunitrazepam and pancuronium. HD1 caused significant ($P < 0.05$) increases in cardiac output and all splanchnic flows. Only hepatic arterial blood flow increased twice as much as did cardiac output. Except for hepatic arterial O_2 delivery, all splanchnic O_2 deliveries decreased. Splanchnic O_2 extractions increased, and O_2 uptakes remained unchanged. There were no changes in mean surface P_{O_2} values or in surface P_{O_2} histograms of liver and small intestine; in portal or hepatic venous *p*H; and in hepatic uptake of pyruvate and lactate. In contrast, during HD2 (despite further increases in flows and O_2 extractions) portal and hepatic venous *p*H decreased; mean surface P_{O_2} of liver and small intestine decreased; and the liver surface P_{O_2} histogram showed broadening and a shift to the left. However, hepatic uptake of lactate and pyruvate, and splanchnic O_2 uptake remained unchanged, and histologic examination did not reveal significant cell injury. These data indicate that in this experimental model limited acute normovolemic HD was well tolerated by the splanchnic organs. After severe HD, gross liver function remained intact, but there was evidence that compensatory mechanisms (increases in flows and O_2 extractions) were no longer fully able to counteract the decrease in splanchnic O_2 delivery. (Key words: Hemodilution: acute normovolemic. Liver: blood flow; histology; metabolism; oxygen supply; oxygen uptake; surface P_{O_2}. Small intestine: blood flow; oxygen uptake; surface P_{O_2}.)

TRANSFUSION carries a multitude of risks, including transfusion reactions, transmission of infection or other diseases, and possible immune suppression.[1] Consequently, alternatives to the use of homologous blood products are increasingly being sought.[2,3] One such alternative is perioperative normovolemic hemodilution (HD).[4]

Numerous studies have investigated the effects of acute HD on individual organs and overall circulation both in humans[5] and in experimental animals.[6] However, there are few data on the effects of HD on splanchnic perfusion and oxygenation.[7–9] Furthermore, neither of the latter studies considered the metabolic or morphologic consequences of an acute decrease in splanchnic arterial O_2 content. Such information may be particularly important because intraoperative liver hypoxia is likely to contribute to postoperative hepatic dysfunction.[10] Since mechanical ventilation and laparotomy by themselves contribute to impaired hepatic circulation,[10,11] avoidance of interventions that significantly interfere with hepatic O_2 supply is particularly important during abdominal surgery. The safety of various degrees of acute HD with regard to liver function therefore needs to be established.

The current study evaluates and compares the effects of limited and severe HD on small intestinal and hepatic perfusion and oxygenation. Since changes in calculated O_2 delivery, O_2 extraction, and surface O_2 tension (P_{O_2}) may not necessarily reflect respective changes in whole organ oxygenation,[12] function, and morphology, the metabolic and histologic parameters indicative of global hepatic function and morphology were determined simultaneously. To our knowledge no such information has yet been available. Although certain strains of pigs have baseline hematocrit (Hct) values less than those of humans, the pig was chosen because of its anatomic and physiologic similarity to humans with respect to the cardiovascular and digestive systems.[13]

Materials and Methods

INSTRUMENTATION

The experimental protocol was approved by the local Committee on Animal Research. The study was performed in 14 4–5-month-old domestic pigs of either sex, weighing 30–38 kg. After overnight fasting and intramuscular azoperon (5 mg/kg), anesthesia was induced with intravenous (iv) methomidate (6 mg/kg) administered *via* ear vein and was maintained by continuous iv

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infusions of ketamine (4 mg · kg^{-1} · h^{-1}) and flunitrazepam (0.0125 mg · kg^{-1} · h^{-1}). After tracheotomy, mechanical ventilation was provided by a constant-volume ventilator (Siemens, SV 900 B, Stockholm, Sweden) and facilitated by a continuous iv infusion of pancuronium (0.12 mg · kg^{-1} · h^{-1}). Respiratory rates and inspired O_2 concentration were adjusted to maintain arterial CO_2 tension between 38 and 42 mmHg, and the arterial O_2 tension between 95 and 115 mmHg. All animals were in the supine horizontal position. Body temperature was continuously monitored by a thermistor of a flow-directed thermodilution catheter (model 93A-131-7F, Edwards Laboratory) and was kept constant by placing the animals on a heating pad and by warming the inspired gases. Catheters were inserted into the abdominal aorta *via* the left and the right femoral artery (16-G Cavafix®-Certo, Braun, Melsungen, FRG), into the pulmonary artery (model 93A-131-7F, Edwards Laboratory), and the superior vena cava *via* right (16-G Cavafix®-Certo, Braun) and left ((7 Fr × 8-inch radiopaque polyurethane two-lumen indwelling catheter, Arrow, Reading, PA) internal jugular veins . All animals received 10–15 ml · kg^{-1} · h^{-1} Ringer's solution to maintain central venous pressure constant.

After median laparotomy, the left hepatic vein was cannulated (18-G Vasofix Braunüle, Braun) *via* transhepatic puncture as previously described.[14] Correct position of the cannula tip in the main stem of the two hepatic veins was verified at autopsy. The portal vein was cannulated (20-G Leader Cath 115, Vygon, Ecouen, France) as previously described.[14]

Precalibrated electromagnetic flow probes (Stölzer Messtechnik, Waldkirch, FRG) of appropriate sizes to ensure a snug fit were placed around the hepatic artery, the portal vein, and the superior mesenteric artery. Care was taken to preserve the periarterial nerve plexus. The flow probes were connected to flow meters with incorporated nonocclusive zero (Hellige, Freiburg, FRG), which was checked repeatedly during the experiment. The superior gastroduodenal artery was ligated to ensure that true hepatic blood flow was measured.

Hemodynamic Measurements

Intravascular catheters (left femoral arterial, portal venous, hepatic venous, and right jugular venous) were connected to pressure transducers (type 840, Senso Nor, Horten, Norway). A multichannel recorder (Hellige, Freiburg, FRG) was used for the recording of signals. Cardiac output was determined by thermodilution technique (cardiac output computer model 404-1, Siemens, Erlangen, FRG). The mean value of triplicate injections of 5 ml ice-cold, temperature-monitored saline was considered to reflect actual cardiac output if the measurements were within a range of ±5% from the calculated mean.

Total hepatic blood flow was calculated as the sum of hepatic arterial and portal venous blood flows. Vascular resistances (systemic, hepatic arterial, portal venous, and superior mesenteric arterial) were calculated using the formulas provided in the appendix. Heart rate was derived from the R–R intervals of an extremity ECG.

Determination of Oxygen Supply/Uptake

Blood gas tensions and *p*H values were determined using an ABL 3 autoanalyzer (Radiometer, Kopenhagen, Denmark). Hemoglobin O_2 saturations were measured by an OSM 3 hemoximeter (Radiometer, Kopenhagen, Denmark). Hct was measured from centrifuged (Bayer AG, Compur Microspin, Leverkusen, FRG) arterial blood sampled in capillary tubes. Hemoglobin concentration was determined by the cyan methemoglobin method. O_2 contents, O_2 deliveries, O_2 uptakes, and O_2 extraction ratios were derived using formulas provided in the appendix.

Preliminary studies showed that there were no differences between portal and mesenteric venous O_2 content. Thus, portal venous O_2 content was used for the calculation of small intestinal O_2 uptake (see appendix).

Determination of Surface P_{O_2} of Liver and Small Intestine

Surface P_{O_2} of liver and small intestine were measured simultaneously using a multiwire platinum electrode, as described previously.[15,16] Preliminary studies showed that placement of the electrodes at different areas of liver and small intestine surfaces produced comparable results. At each stage a total of about 100 individual P_{O_2} measurements were obtained at 10–15 different electrode locations. The distribution of these values, presented as summary surface P_{O_2} histograms, reflects tissue oxygenation, which is the net result of (*i.e.*, the difference between) nutritive blood flow and tissue O_2 consumption.[17]

Determination of Hepatic Metabolic Function

Arterial, portal venous, and hepatic venous blood samples were collected for enzymatic determination[18] of lactate and pyruvate concentrations. Hepatic uptake of lactate and pyruvate were calculated using the formulas provided in the appendix.

Arterial glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were measured using optimized standard methods as recommended by the German Society of Clinical Chemistry.[19]

Histologic Examinations of the Liver

In 12 animals liver tissue samples were obtained by standard needle biopsy (cutting biopsy needle 1.8 mm

× 10 cm, Angiomed, Karlsruhe, FRG). Sections for light microscopy were fixed in a solution containing 1% formaldehyde, 0.5% glutaraldehyde, and 0.1 M calcium acetate were stained with hematoxylin–eosin and van Gieson's stain. Cell damage was estimated semiquantitatively, as to the presence of no necrosis (−), single cell necroses (+), and larger centrolobular necroses (++).

In order to reduce bias, slides were assessed by two independent observers unaware of the experimental procedure during which the biopsies had been obtained. In addition, part of the slides were evaluated twice by both observers with the results of the first assessment unknown to both.

For electron microscopy the specimens were fixed in 3.5% buffered glutaraldehyde (*p*H 7.4), postfixed in 1% osmium tetroxide for 3 h, and embedded in Araldit. Ultrathin sections were contrasted with 2% uranyl acetate and 0.02% lead citrate and examined using a Philipps CM 10 electron microscope.

Experimental Protocol

At the end of the surgical preparation, at least 30 min was allowed before baseline readings during ketamine/flunitrazepam anesthesia were obtained.

Subsequently, two degrees of normovolemic HD were induced: these were limited HD (mean Hct 20%, "HD1") and severe HD (mean Hct 14%, "HD2"). This was achieved by simultaneously replacing blood (withdrawn *via* the right femoral artery) by roughly equal amounts of 6% hydroxyethyl starch (molecular weight 450,000 D, Plasmasteril®, Fresenius, Homburg, FRG) administered at body temperature *via* the left jugular vein. As indices of normovolemia, central venous and pulmonary capillary

TABLE 1. Baseline Values and Values during Limited (HD1) and Severe (HD2) Hemodilution

Variables	Baseline	HD1	HD2
Hematocrit (%)	30 ± 1	20 ± 1*†	14 ± 1*†
Hemoglobin (g%)	9.1 ± 0.4	6.0 ± 0.3*†	4.1 ± 0.2*†
Intravascular pressure			
Central venous (mmHg)	2.5 ± 0.4	2.5 ± 0.4	2.6 ± 0.4
Pulmonary capillary wedge (mmHg)	6.7 ± 0.4	6.6 ± 0.5	6.7 ± 0.5
Portal venous (mmHg)	7.4 ± 0.6	7.1 ± 0.8	7.5 ± 0.9
Hepatic venous (mmHg)	2.7 ± 0.4	2.6 ± 0.4	2.8 ± 0.4
Blood flows			
Cardiac output (l/min)	3.6 ± 0.2	4.1 ± 0.2*†	4.5 ± 0.2*†
Total hepatic (ml/min)	652.7 ± 20.4	793.6 ± 28.6*†	895.4 ± 32.9*†
Hepatic arterial (ml/min)	87.2 ± 7.6	136.0 ± 8.9*†	165.7 ± 13.4*†
Hepatic arterial (% of total hepatic)	13 ± 1	17 ± 2*†	19 ± 2*†
Portal venous (ml/min)	576.4 ± 27.5	674.8 ± 29.8*†	750.4 ± 43.8*†
Superior mesenteric arterial (ml/min)	392.9 ± 33.6	447.4 ± 51.8*†	498.3 ± 51.9*†
Vascular resistances			
Hepatic arterial (U)	1041 ± 148	705 ± 47*†	503 ± 37*†
Portal venous (U)	9 ± 1	7 ± 1*†	7 ± 1*†
Superior mesenteric arterial (U)	224 ± 25	201 ± 29*†	173 ± 35*†
Hemoglobin oxygen saturation			
Mixed venous (%)	80.2 ± 2.1	75.5 ± 1.7*†	67.0 ± 4.5*†
Oxygen content			
Mixed venous (ml/100 ml)	10.1 ± 0.7	6.2 ± 0.4*†	3.9 ± 0.4*†
Oxygen extractions			
Liver	0.30 ± 0.03	0.38 ± 0.04*†	0.49 ± 0.04*†
Small intestine	0.21 ± 0.02	0.28 ± 0.04*†	0.33 ± 0.03*†
Total body	0.19 ± 0.02	0.24 ± 0.02*†	0.32 ± 0.05*†
Tissue surface P_{O_2}			
Liver (mmHg)	55.3 ± 2.9	54.0 ± 1.4	40.9 ± 5.4*†
Small intestine (mmHg)	55.7 ± 2.4	53.9 ± 1.1	45.4 ± 2.7*†
Hepatic uptakes			
Pyruvate (μmol/min)	10 ± 5	23 ± 16	14 ± 8
Lactate (μmol/min)	672 ± 162	483 ± 102	558 ± 165
*p*H values			
Arterial	7.35 ± 0.01	7.34 ± 0.02	7.32 ± 0.02
Portal venous	7.30 ± 0.01	7.29 ± 0.02	7.25 ± 0.02*†
Hepatic venous	7.33 ± 0.02	7.33 ± 0.02	7.30 ± 0.02*†
Liver enzyme concentrations			
GOT (U/l)	33 ± 4	26 ± 2*†	20 ± 2*†
GPT (U/l)	24 ± 3	15 ± 2*†	9 ± 2*†

Values are means ± SEM.
* $P < 0.05$ compared to baseline.
† $P < 0.05$ compared to HD1.
¶ GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase.

wedge pressures were kept constant during the HD procedure.

After the Hct had been decreased to the required levels of 20% (HD1) and 14% (HD2), the respective measurements at HD1 and HD2 were made after at least 15 min of stabile hemodynamics.

In five additional animals, the effects of time on the stability of the surgical preparation were evaluated during baseline conditions (laparotomy and ketamine/flunitrazepam anesthesia). The surgical preparation was performed as described above. No intervention was undertaken after baseline readings had been obtained, and repeat measurements were made 3 h later.

In seven animals biopsies were obtained approximately 30 min after the end of the surgical preparation (baseline) and after severe HD (HD2). In three animals biopsies were taken at baseline and 3 h later (time controls). In order to evaluate the effects of the surgical preparation itself, in five animals liver biopsies were obtained immediately after laparotomy, and also after the entire instrumentation had been finished.

Statistical Analysis

The data were statistically analyzed by Friedman's statistic followed by Wilcoxon signed-rank test (for comparison between experimental periods) and by Kruskal-Wallis one-way analysis of variance followed by Mann-Whitney test (for comparisons within experimental periods). A P value of <0.05 was considered statistically significant. Values are presented as means ± standard errors of the means (SEM).

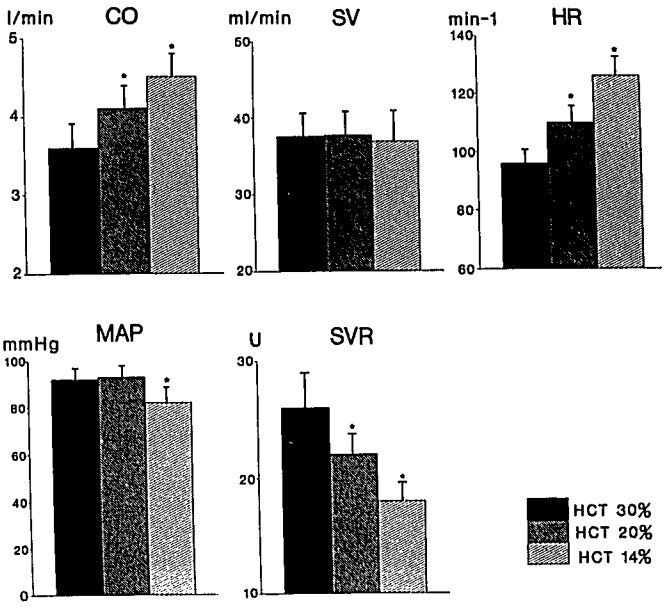

FIG. 1. Systemic hemodynamics during baseline (Hct 30%) and during limited (Hct 20%) and severe (Hct 14%) acute normovolemic hemodilution. *$P < 0.05$ compared to preceding value. CO = cardiac output; SV = stroke volume; HR = heart rate; MAP = mean arterial pressure; SVR = systemic vascular resistances; HCT = hematocrit.

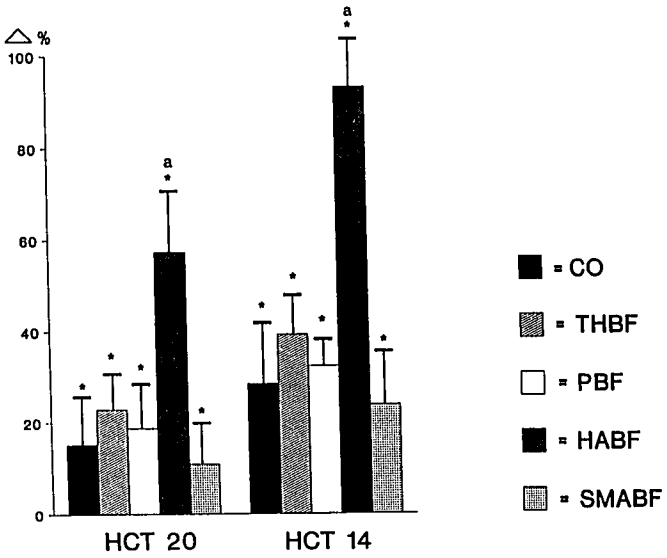

FIG. 2. Percent changes from baseline values (see table 1 and fig. 1) in cardiac output (CO) and splanchnic blood flows. *$P < 0.05$ compared to no (zero) change. a = $P < 0.05$ compared to all other blood flows; THBF, HABF, PBF, and SMABF = total hepatic, hepatic arterial, portal, and superior mesenteric arterial blood flows, respectively.

Results

In order to reduce the Hct from an initial value of 30% ± 1 to 20 ± 1 (HD1) and 14% ± 1 (HD2), 17 ± 2 (HD1) and 35 ± 2 ml/kg (HD2) of blood were withdrawn and replaced by 18 ± 1 (HD1) and 38 ± 2 ml/kg (HD2) of 6% hydroxyethyl starch. At this exchange ratio central venous and capillary wedge pressures remained unchanged (table 1).

Systemic and Splanchnic Hemodynamics

Stepwise normovolemic HD resulted in approximately 15% (HD1) and 30% (HD2) increases in cardiac output and heart rate (table 1, fig. 1). Stroke volume remained unchanged, and mean arterial pressure decreased slightly (11%) during HD2 only (fig. 1). Portal venous and hepatic venous pressures remained unchanged during both HD steps (table 1).

Increasing HD induced progressive increases in total hepatic, hepatic arterial, portal venous, and superior mesenteric arterial blood flows (table 1). When expressed as percent changes from baseline values, hepatic arterial blood flow increased more than twice as much as did cardiac output and all other blood flows during each HD step (fig. 2). As a consequence, hepatic arterial blood flow expressed as fraction of total hepatic blood flow increased significantly (table 1). Similarly, whereas total hepatic

(19% ± 1) and superior mesenteric arterial blood (12% ± 1) flows expressed as fractions of cardiac output did not change, hepatic arterial blood flow expressed as a fraction of cardiac output increased from the initial 2.2% ± 0.3 to 3.5% ± 0.2 during HD2.

There also were quantitative differences in the effects of HD on regional vascular resistances. Portal venous vascular resistance decreased initially but was not decreased further by HD2. In contrast, all other calculated vascular resistances (systemic, hepatic arterial, and superior mesenteric arterial) decreased progressively (fig. 1, table 1). Again, the greatest decrease in vascular resistance was observed in the hepatic arterial bed (approximately 30 and 50% during HD1 and HD2, respectively) (table 1).

There were no correlations between increases in hepatic arterial blood flow or decreases in hepatic arterial vascular resistances and changes in hepatic venous or portal venous P_{O_2} and O_2 saturation.

Systemic and Splanchnic O_2 Supply/Uptake Relationships

Increasing normovolemic HD resulted in progressive decreases in arterial, portal venous, hepatic venous, and mixed venous O_2 contents. This was due both to progressive reduction in Hct and (except for arterial hemoglobin O_2 saturation, which remained unchanged) decreases in O_2 saturations (portal, hepatic, and mixed venous) (fig. 3, table 1).

Consequently, except for hepatic arterial O_2 delivery, which was preserved during both HD stages, all O_2 deliveries (total hepatic, portal venous, and superior mesenteric arterial) decreased progressively (fig. 3).

O_2 delivery/uptake relationships associated with limited and severe normovolemic HD are shown in figure 4. Whole body, liver, and small intestine O_2 uptakes remained unchanged and were independent of O_2 deliveries during both HD stages. All O_2 extraction ratios increased continuously (table 1).

Tissue Oxygenation of Liver and Small Intestine

There were no differences in mean liver and small intestine surface P_{O_2} between baseline values and those obtained during limited (HD1) normovolemic HD (figs. 5, 6, table 1). However, severe HD (HD2) resulted in decreases in mean liver and small intestine surface P_{O_2} values of approximately 30 and 20%, respectively. Summary surface P_{O_2} histograms of liver and small intestine did not change their original bell shape during HD1. However, during HD2 the liver surface P_{O_2} histogram was broadened at the base and shifted to the left, with P_{O_2} values below 20 mmHg, in approximately 20% of all P_{O_2} measurements. In contrast, the small intestine surface P_{O_2} histogram retained its bell-shaped form even during HD2, with no P_{O_2} values in the hypoxic ranges.

Hepatic Metabolic Function

Hepatic lactate and pyruvate uptake remained unchanged during both HD1 and HD2 (table 1). Whereas

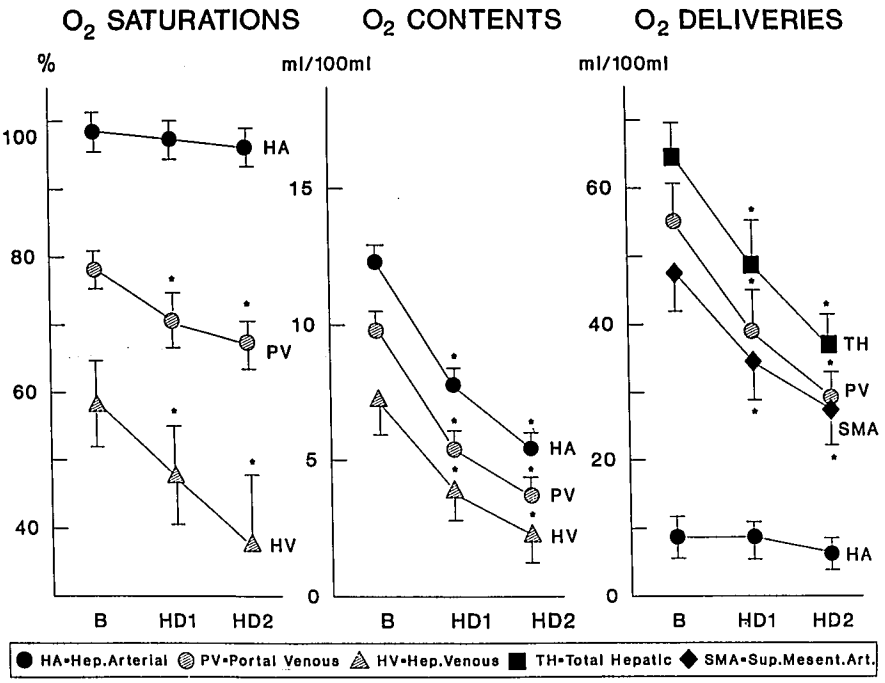

FIG. 3. Hemoglobin O_2 saturations, contents, and deliveries during baseline (B) and during limited (HD1) and severe (HD2) normovolemic hemodilution. *$P < 0.05$ compared to preceding value.

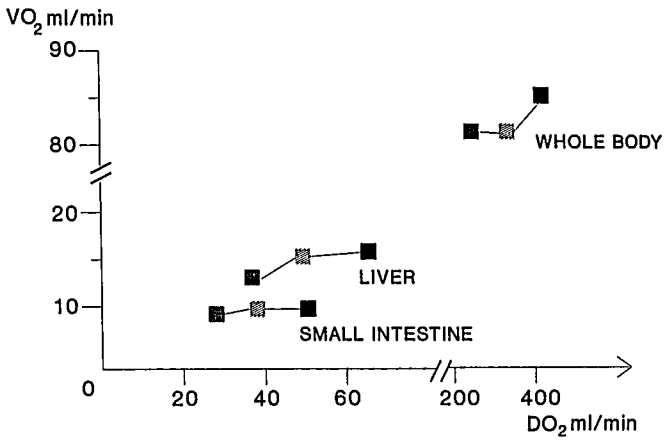

FIG. 4. Relationship between O_2 uptake ($\dot{V}_{O_2}$) and O_2 delivery ($\dot{D}_{O_2}$) of whole body, liver, and small intestine during baseline (hematocrit [HCT] 30%, solid squares) and during limited (HCT 20%, dark hatched squares) and severe (HCT 14%, light hatched squares) normovolemic hemodilution.

arterial *p*H remained unchanged during both HD1 and HD2, portal venous and hepatic venous *p*H values remained unchanged during HD1 only, but they decreased during HD2. There were continuous decreases in serum concentrations of GOT and GPT (table 1).

Histopathology of the Liver

At baseline, in three of seven animals studied there was no evidence of cell injury. Four animals demonstrated single-cell necroses. After severe HD, all animals showed single-cell necroses. Differences between baseline and HD2 were not statistically significant. Histology was not affected by either time or the surgical preparation.

After severe HD, four of seven animals showed vacuolization of hepatocytes predominantly localized in the midlobular area. On osmication these vacuoles were found to be osmiophobic (and so were confirmed as not containing fat). Ultrastructural investigation revealed large vacuoles occupying large parts of the cytoplasm. The vacuoles were lined by a single-unit membrane and contained a delicate flocculent substance. Sometimes short linear or spiral membrane fragments could be observed. These large vacuoles resulted from fusion of smaller endocytotic vesicles. Other cell organelles, in particular mitochondria and the endoplasmic reticulum appeared normal. Kupffer cells, Ito cells, and sinusoidal endothelial cells remained unchanged (fig. 7).

Effect of Time on the Stability of the Surgical Preparation during Baseline Anesthesia

In the five animals studied, changes in parameters of hemodynamics, oxygenation, and metabolism over a 3-h period were not statistically significant. Except for total systemic (22% increase) and total hepatic O_2 uptake (20% decrease) and hepatic lactate uptake (35% decrease), changes in mean values of 45 parameters measured or calculated did not exceed 13%.

In three animals studied there were only insignificant changes in liver biopsies over the 3-h period.

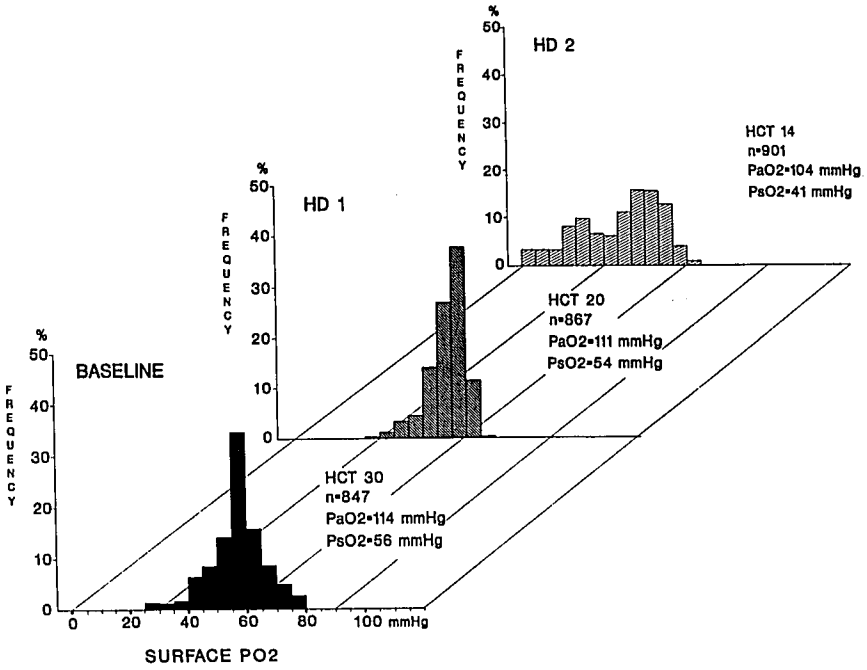

FIG. 5. Summary histograms of liver surface P_{O_2} from nine pigs during baseline and during limited (HD1) and severe (HD2) normovolemic hemodilution (HD). n = total number of measurements; Ps_{O_2} = mean surface tissue P_{O_2}; and Pa_{O_2} = mean arterial P_{O_2} during recording of the histograms.

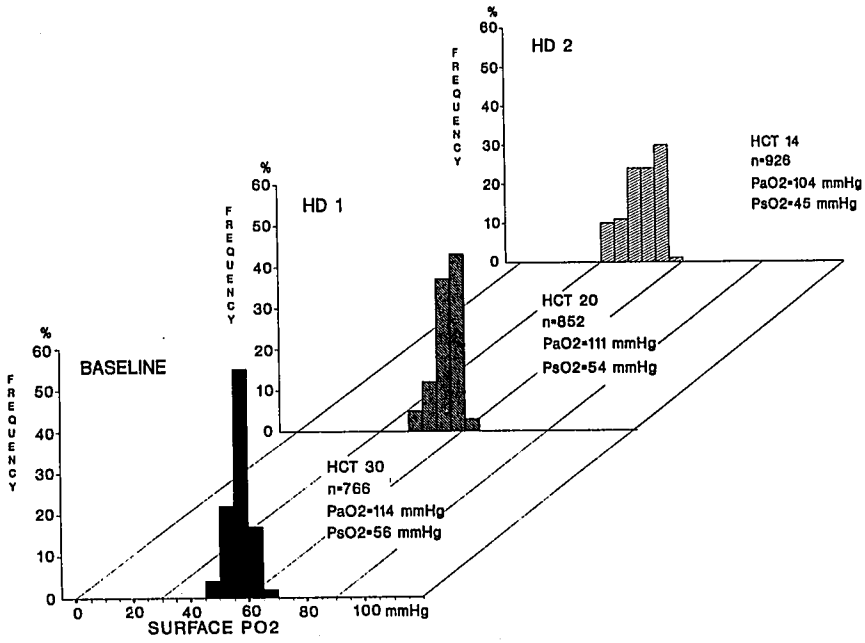

FIG. 6. Summary histograms of small intestine surface P_{O_2} (n = 9). (See legend to fig. 5 for abbreviations.)

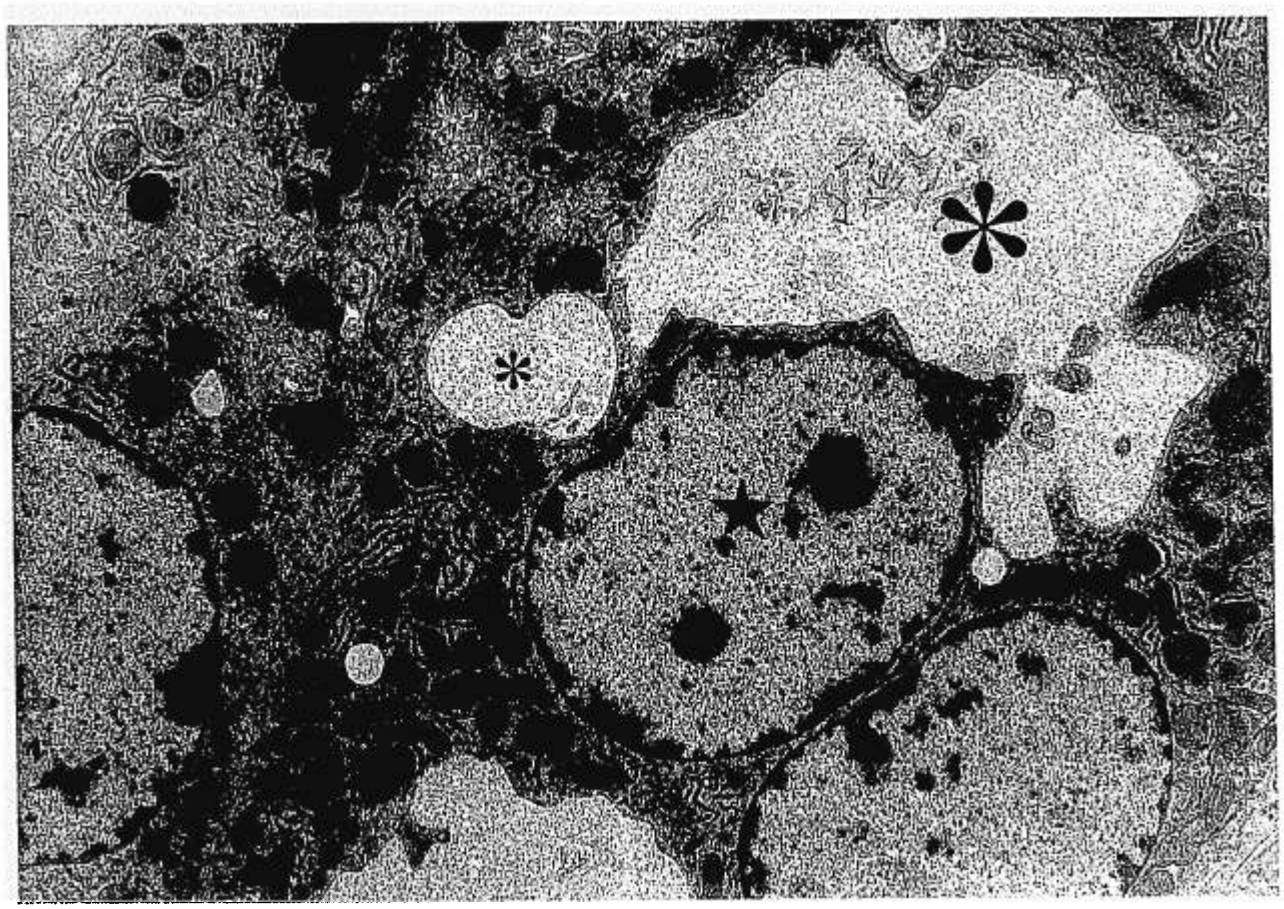

FIG. 7. Midzonal hepatocyte after severe hemodilution with hydroxyethyl starch. Hydroxyethyl starch-containing, irregular-shaped giant endosome (*large asterisk*), lined by a single unit membrane and containing delicate flocculent substance. The giant endosome occupies large parts of the cytoplasma. Small endosome before fusion (*small asterisk*). Mitochondria (*arrowhead*) revealed no swelling. Endoplasmatic reticulum (*double arrowhead*) showed no dilatation. Nucleolus (*star*). Original magnification ×4,900.

Effect of Surgical Procedure on Liver Morphology

In the five animals studied liver biopsies obtained immediately after laparotomy and after the entire instrumentation did not differ significantly from each other.

Discussion

The principal findings of this study can be summarized as follows. 1) Progressive HD resulted in progressive increases in all splanchnic flows. 2) Hepatic arterial blood flow increased out of proportion to all other splanchnic flows and cardiac output. 3) No hepatic arterial buffer response was noted during acute HD. 4) During limited HD (decrease in Hct from 30 to 20%) mean surface P_{O_2} of liver and small intestine, and portal and hepatic venous *p*H were maintained. 5) During severe HD (decrease in Hct to 14%), the surface P_{O_2} of liver and small intestine declined; the surface P_{O_2} histograms shifted to the left; and portal and hepatic venous *p*H values decreased. 6) Even during severe HD, splanchnic O_2 uptake had not yet become dependent on O_2 delivery.

Critique of Methods

These studies were conducted in anesthetized pigs whose lungs were ventilated during laparotomy. The pig was chosen because of its anatomic and physiologic similarities to humans with respect to the cardiovascular and digestive systems,[13] and particularly with respect to liver enzyme systems.[20] Both laparotomy and mechanical ventilation are known to interfere with splanchnic perfusion.[10,11] Both ketamine[21] and pancuronium[22] have little or no effect on liver and preportal circulation. The effects of flunitrazepam on the splanchnic circulation are unknown. It therefore is emphasized that the effects associated with acute HD occurred during a baseline anesthesia with ketamine/flunitrazepam and during laparotomy. In addition, it should be emphasized that animals with a presumably normal circulation were studied. Translation of this data to patients with diseased circulation (*e.g.*, longstanding hypertension or atherosclerosis) is therefore unwarranted.

The protocol required extensive surgical preparation. Both baseline anesthesia and surgery may have resulted in spontaneous deterioration of the preparation over time and thus may have influenced the results. However, findings of insignificant changes in parameters of hemodynamics, oxygenation, and metabolism as well as in liver morphology over time obtained in numerous control animals rule out significant spontaneous deterioration in this experimental model.

Six percent hydroxyethyl starch was chosen as replacement fluid. It is a macromolecular polymer consisting of hydroxyethylated glucose molecules linked by α-1.4 bonds. The intravascular half-life is about 12 h. Due to its physicochemical characteristics and minimal adverse effects, it is as suitable as 6% Dextran 70 to induce HD.[23]

If potentially adverse effects of acute HD are to be kept at a minimum, maintenance of normovolemia becomes essential. Although we did not determine actual blood volume, circumstantial evidence suggested that normovolemia was maintained. First, the amount of hydroxyethyl starch administered was slightly in excess of the amount of blood withdrawn. Second, right and left cardiac filling pressures remained unchanged throughout. Third, cardiac output was able to increase by 15 (HD1) and 25% (HD2). Fourth, stroke volume remained unchanged despite increases in heart rate by 15 (HD1) and 30% (HD2).

The technique of measuring surface P_{O_2} using O_2-sensitive multiwire electrodes has been well established.[15,16] Although surface P_{O_2} may not necessarily reflect whole organ P_{O_2}, this technique allows determination of tissue P_{O_2} without causing tissue trauma or interference with the microcirculation.[16]

Hemodilution and Systemic Circulation

In agreement with previous work,[9,24,25] cardiac output increased in response to HD. However, in contrast to previous findings, this increase was due to an increase in heart rate rather than in stroke volume. Differences in heart rate response may be related to differences in species, baseline anesthesia, cardiac filling pressures, and baseline heart rates. It is not at all surprising that heart rates are less likely to increase in response to HD if they are already elevated during baseline conditions.[26] It is unlikely that the increase in heart rate reflected hypovolemia. If hypovolemia had been present, stroke volume would have decreased in response to the increase in heart rate; cardiac output would not have increased by 15 (HD1) and 25% (HD2); and right and left cardiac filling pressures probably would not have remained unchanged throughout.

Hemodilution and Splanchnic Circulation

Progressive HD resulted in progressive increases in all flows. Whereas most flows (*i.e.*, superior mesenteric arterial, portal venous, and total hepatic) increased proportionally to cardiac output (fig. 2), hepatic arterial blood flow increased twice as much. Thus, the increase in hepatic arterial blood flow cannot be explained solely by the increase in cardiac output or the decrease in blood viscosity caused by HD. These findings imply that the hepatic artery (more than other vascular beds and apparently unrelated to or in addition to the changes in cardiac output and viscosity) actively dilated.

If active hepatic arterial vasodilation was to compensate for the decrease in O_2 delivery, what might have been

the stimulus? Hepatic venous P_{O_2} and O_2 saturations declined progressively during HD1 and HD2, and hepatic venous *p*H decreased during HD2. However, there were no statistically significant correlations between changes in hepatic arterial blood flow or hepatic vascular resistance and changes in hepatic venous P_{O_2} O_2 saturation, or *p*H.

The pronounced increases in hepatic arterial blood flow observed in this study could not necessarily have been expected. The hepatic arterial buffer response[27] predicts that changes in portal blood flow lead to opposite changes in hepatic arterial blood flow. Accordingly, as portal blood flow increases, hepatic arterial blood flow decreases. Numerous investigations have documented the hepatic arterial buffer response,[28,29] suggesting that total hepatic perfusion is flow-regulated rather than being demand-dependent. It is postulated that the rate of intrahepatic adenosine washout forms the basis for the hepatic arterial buffer response.[30]

The current findings must be interpreted in this context. Either they provide evidence against the adenosine-washout hypothesis (*i.e.*, total hepatic perfusion is not just flow-regulated), or they indicate that factors related to HD overcome or at least modulate the hepatic arterial buffer response. Two such factors may be the associated changes in cardiac output and blood viscosity. However, this does not explain why hepatic arterial vascular resistance declined almost twice as much as did the resistances of other vascular beds. At least after HD1, there was no evidence that hepatic oxygenation was endangered. We are unable to provide a plausible explanation for why hepatic arterial blood flow increased out of proportion to other flows. It is conceivable that the hepatic arterial buffer response is not as effective in pigs as it is in cats[30] or dogs.[29] It also is possible that the degree of the response varies with the baseline anesthesia and with the initial value for the relative contribution of hepatic arterial blood flow to total hepatic blood flow.

Hemodilution and Splanchnic Oxygenation, Hepatic Metabolism, and Morphology

As a result of the progressive decrease in Hct and due to decreases in O_2 saturations (portal and hepatic venous), there were progressive decreases in O_2 contents of all vascular beds (fig. 3). Consequently, O_2 deliveries to most vascular beds (total hepatic, portal venous, superior mesenteric arterial, and systemic) decreased (fig. 3) despite concomitant increases in respective flows. The hepatic artery was the only vascular bed that maintained O_2 delivery because of its unchanged O_2 saturation (fig. 3) and the out-of-proportional increase in flow. Since in this model hepatic arterial blood flow contributes between just 13 (baseline) and 19% (HD2) to total hepatic blood flow, unchanged hepatic arterial O_2 delivery could not compensate for the marked decrease in portal venous O_2 delivery (as reflected by the decrease in total hepatic O_2 delivery). The pig compensated for reduced O_2 contents and O_2 deliveries by progressively increasing flows (by lowering vascular resistances) and O_2 extraction ratios.

After the first HD step, there was no evidence of organ dysfunction. Portal as well as hepatic venous *p*H values remained unchanged, as did the distribution and the mean values of surface P_{O_2} values of liver and small intestine. Similarly, hepatic uptakes of lactate and pyruvate did not change significantly.

In contrast, after the second HD step there were significant decreases in both portal (from 7.29 to 7.25) and hepatic venous *p*H values (from 7.33 to 7.30), and the summary P_{O_2} histogram of the liver showed a broadening at the base and a leftward shift, with more than 25% of P_{O_2} values below 25 mmHg. This is clear evidence of impaired microcirculation. Although a tendency for a leftward shift also was observed in the surface P_{O_2} histogram of the small intestine, these changes were less pronounced: no P_{O_2} values were below 30 mmHg. On the other hand, the decreases in mean surface P_{O_2} values were similar for liver (from 54 to 41 mmHg) and small intestine (from 54 to 41 mmHg). Combined evidence thus suggests that during severe HD (mean Hct 14%), compensatory mechanisms (*i.e.*, increases in flows and O_2 extraction ratios) were no longer able fully to compensate for the decreases in O_2 deliveries and to maintain tissue oxygenation at baseline values.

Despite decreases in O_2 delivery by approximately 40% and evidence of just marginally compensated organ function, O_2 uptake of liver and small intestine did not decrease significantly. This implies that the point at which tissue O_2 uptake becomes limited by delivery in a supply-dependent manner (*i.e.*, critical O_2 delivery) had not yet been reached. However, plots of O_2 uptake *versus* delivery of both liver and small intestine (fig. 4) suggest that between data points HD1 and HD2, O_2 uptake may be about to decline; *i.e.*, the point of critical O_2 delivery may almost have been reached.

After severe HD large vacuoles were observed in the midzonal hepatocytes in four of seven animals. Ultrastructural investigation revealed giant endosomes containing a delicate flocculent substance. Clearly, these were caused by an endocytotic process leading to a fusion of small endosomes. In accordance with previous reports,[31] we assume that these vacuoles reflect an early hepatocytic storage of hydroxyethyl starch. Interestingly, these phenomena were not observed in all animals, indicating differences between individuals in the ability of hepatocytes to uptake hydroxyethyl starch. It is important that even in these midzonal hydroxyethyl starch-storing hepatocytes, no signs of hypoxic damage, *i.e.*, swelling of mitochondria, dilation of the endoplasmic reticulum, or fatty

degeneration were observed. So, it seems unlikely that the observed storage of hydroxyethyl starch impaired hepatocellular function.

Comparison with Previous Work

Most previous studies have found insignificant changes in hepatic arterial blood flow in response to HD.[8,9,25,32] Only one study[7] in addition to ours has shown a significant increase in hepatic arterial blood flow. Total hepatic blood flow was found to be unchanged[8] or to increase[7] and portal blood flow also to increase.[8]

Direct comparison of these results with ours is difficult because of differences in species (pigs *vs.* cats[8] *vs.* dogs[7,9,25] *vs.* rats[32]), in baseline anesthesia (ketamine/flunitrazepam *vs.* pentobarbital[8,9,25] *vs.* neuroleptic[32] *vs.* conscious[7]), in degree of HD, in means of achieving HD, and in methods of determining hepatic blood flow (electromagnetic flow probes *vs.* microspheres[9,25,32] *vs.* bromsulphalein[7] *vs.* reservoir[8]). Furthermore, in most of these studies portal blood flow was not determined.[7,9,25,32] Such lack of flow measurement will render determination of total hepatic blood flow and consequently any evaluation of a potential hepatic arterial buffer response impossible.

Conclusions

In conclusion, we have shown that in anesthetized and laparotomized pigs, acute moderate HD (from an initial Hct of 30 down to 20%) with 6% hydroxyethyl starch is well tolerated by liver and small intestine. Compensatory mechanisms (increases in splanchnic flows and O_2 extractions) effectively counteracted the decreases in splanchnic O_2 deliveries. It is interesting to note that hepatic arterial blood flow increased twice as much as cardiac output, superior mesenteric arterial flow, and portal blood flow.

During further HD (to a final mean Hct of 14%), lack of statistically significant changes in splanchnic O_2 uptake, in hepatic metabolism (lactate and pyruvate uptake), and in hepatic histology indicate that even severe HD did not have grossly adverse effects. However, significant decreases in mean surface P_{O_2} of liver and small intestine; a broadening and a leftward shift of the liver surface P_{O_2} histogram; decreases in portal and hepatic venous *p*H and O_2 content values; and tendency for hepatic cellular injury, all provide evidence that compensatory mechanisms were becoming exhausted. However, lack of swelling of mitochondria, lack of dilation of the endoplasmic reticulum, and lack of fatty degeneration exclude injury at the ultrastructural level.

It remains to be determined whether the human splanchnic circulation and organs respond to acute HD in a manner similar to those of the pig. Normal Hct values in humans are approximately 45% (rather than 30% as in the pig). We are therefore unable to define the human correlate to a Hct of 20 (after HD1) and 14% (after HD2) in the pig. In the human, comparably low Hct values may reflect a much greater degree of HD. If only the percent changes in Hct after HD1 (33%) and HD2 (50%) were applied, this would correspond to absolute Hct values of approximately 30 (HD1) and 20% (HD2) in humans.

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Appendix: Formulas

Vascular Resistances

$$SVR(U) = \frac{MAP\ (mmHg) - CVP\ (mmHg)}{CO\ (l/min)}$$

$$HAVR(U) = \frac{MAP\ (mmHg) - HVP\ (mmHg)}{HABF\ (ml/min)} \times 10^3$$

$$PVVR(U) = \frac{PVP\ (mmHg) - HVP\ (mmHg)}{PBF\ (ml/min)} \times 10^3$$

$$SMAVR(U) = \frac{MAP\ (mmHg) - PVP\ (mmHg)}{SMABF\ (ml/min)} \times 10^3$$

SVR, HAVR, PVVR, and SMAVR = systemic, hepatic arterial, portal venous, and superior mesenteric arterial vascular resistances, respectively; MAP, CVP, PVP, and HVP = mean arterial, central venous, portal venous, and hepatic venous pressures, respectively; CO = cardiac output; HABF, PBF, and SMABF = hepatic arterial, portal venous, and superior mesenteric arterial blood flows, respectively.

Oxygen Supply/Uptake

$$O_2\ content\ (C_{O_2}) = Hb \times O_2\ saturation \times 1.34 + P_{O_2} \times 0.0031$$

$$O_2\ delivery\ (\dot{D}_{O_2}) = O_2\ content \times flow$$

$$\dot{D}_{O_2}TH = \dot{D}_{O_2}HA + \dot{D}_{O_2}PV$$

$$\dot{D}_{O_2}HA = C_{O_2}A \times HABF \times 10^{-2}$$

$$\dot{D}_{O_2}PV = C_{O_2}PV \times PVF \times 10^{-2}$$

$$\dot{D}_{O_2}SMA = C_{O_2}A \times SMABF \times 10^{-2}$$

$$\dot{V}_{O_2}TH = (C_{O_2}PV - C_{O_2}HV) \times PBF \times 10^{-2} + (C_{O_2}A - C_{O_2}HV) \times HABF \times 10^{-2}$$

$$\dot{V}_{O_2}SI = (C_{O_2}A - C_{O_2}PV) \times SMABF \times 10^{-2}$$

$$E_{O_2}TH = \dot{V}_{O_2}TH/\dot{D}_{O_2}TH$$

$$E_{O_2}SI = \dot{V}_{O_2}SI/\dot{D}_{O_2}SMA$$

$\dot{D}_{O_2}TH$, $\dot{D}_{O_2}HA$, $\dot{D}_{O_2}PV$, and $\dot{D}_{O_2}SMA$ = total hepatic, hepatic arterial, portal venous, and superior mesenteric arterial O_2 deliveries, respectively; $C_{O_2}A$, $C_{O_2}PV$, and $C_{O_2}HV$ = systemic arterial, portal venous, and hepatic venous O_2 contents, respectively; $\dot{V}_{O_2}TH$ and $\dot{V}_{O_2}SI$ = total hepatic and small intestinal O_2 uptake, respectively; $E_{O_2}TH$ and $E_{O_2}SI$ = total hepatic and small intestinal O_2 extraction ratios, respectively.

Hepatic Metabolism

$$Heptaic\ lactate\ uptake = (C_{LAC}PV - C_{LAC}HV) \times PBF + (C_{LAC}HA - C_{LAC}HV) \times HABF$$

$$Hepatic\ pyruvate\ uptake = (C_{PYR}PV) - C_{PYR}HV) \times PBF + (C_{PYR}HA) - C_{PYR}HV) \times HABF$$

C_{LAC} and C_{PYR} = lactate and pyruvate concentrations, respectively, in hepatic arterial (HA), portal venous (PV), and hepatic venous (HV) blood.