Determination of Minimal Hemoglobin Level Necessary for Normothermic Porcine Ex Situ Liver Perfusion

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Background. In current studies of ex situ liver perfusion there exists considerable variability in perfusate composition, including the type of oxygen carrier. Herein, we aim to clarify the minimal hemoglobin level necessary during normothermic porcine ex situ liver perfusion. **Methods.** Livers procured from 35 to 45 kg domestic pigs were connected to our experimental ex situ circuit (n = 10). In the treatment group, perfusate was sequentially diluted hourly to predetermined hemoglobin levels. At the end of each hemoglobin dilution, perfusate samples were analyzed for liver transaminases, lactate dehydrogenase (LD), total bilirubin, and lactate levels. Liver oxygen consumption was measured. In the control group, livers were perfused continually for a duration of 24 hours at target hemoglobin levels of 30 and 20 g/L. **Results.** Rising liver transaminases, significantly higher lactate ($P < 0.001$), and LD levels ($P < 0.001$) were noted at lower perfusate hemoglobin levels in the treatment group. Liver oxygen utilization ($P < 0.001$) and hepatic artery oxygen delivery ($P < 0.001$) were significantly lower at lower hemoglobin levels, whereas liver vessel resistance remained relatively constant. Histology demonstrated increasing parenchymal damage at lower hemoglobin levels. In control livers, higher perfusate transaminases, higher lactate, and LD levels were noted at a perfusion hemoglobin level of 20 g/L. **Conclusions.** Ex situ liver function decompensated during perfusion between a mean hemoglobin level of 30 to 20 g/L, as evidenced by notably rising lactate and LD levels. This study demonstrates optimal hemoglobin concentration during normothermic ex situ liver perfusion to ensure a fully metabolically functioning graft.

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Ex situ liver perfusion has been repeatedly suggested as a way to resuscitate marginal and extended criteria liver grafts, thereby improving the quality and possibly the quantity of transplanted livers. With the worldwide shortage of organs available for transplantation, and the increased use of marginal and extended donor organs, the current approach of cold static preservation has declared its limitations.[1,2] As a result, in recent years, interest in achieving optimization in the field of ex situ organ perfusion has increased significantly. Among the different modalities, normothermic ex situ liver perfusion shows the most theoretical promise to facilitate dynamic assessment of organ viability before transplantation, with a suitable oxygen carrier to meet the metabolic demands of the liver.[3,4] Recent publication of a large multicenter randomized trial of ex situ normothermic liver perfusion reported significant reductions in graft injury, even after accounting for a significantly reduced rate of organ discard as well a longer mean preservation time as compared to static cold storage (SCS).[5] This landmark publication strongly demonstrates the utility of this technology in future clinical practice, as well as the prescient interest and need for further investigations to optimize its translational potential.

There currently exists remarkable variability in ex situ circuit design, ex situ perfusate composition, as well as surrogate viability measures used to evaluate liver function. Liver perfusion circuits differ predominantly in whether there are 1 or 2 perfusion pumps, whether perfusion is pulsatile or continuous, substrate additives, and the temperature of the circulating volume.[3,6-10] The perfusates within the circuits differ in the composition of the priming base, as well as the concentration and type of the oxygen carrier.[11-13]

In both experimental and clinical normothermic ex situ liver perfusion, most groups use an erythrocyte-based oxygen carrier; however, it has not been demonstrated what minimal

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hemoglobin level is sufficient to meet the needs of a fully-functioning liver graft. Perfusate hemoglobin concentrations are seldom reported, and are often well below species appropriate normal levels (Table 1).

More recently, novel acellular oxygen carriers have been investigated as alternatives to blood based carriers, with promising results. These products offer a number of theoretical advantages, including eliminating blood born infection risk, lower immunogenic reactivity, simplified logistics and a longer shelf life, the ultimate advantages of which remain to be determined.[28-30] Nevertheless, most groups currently use erythrocytes in normothermic machine perfusion (NMP) and we therefore sought to investigate what level of hemoglobin would be optimal to maintain graft viability.

The hemoglobin levels chosen in the experimental perfusions were on the lower spectrum of what would likely be physiologically feasible to sustain a liver over the duration of NMP. Blood products are a scarce resource, and the clinical implication of perfusions performed with lower hemoglobin levels, in the absence of alternatives, simplifies logistics and improves resource utilization.

MATERIALS AND METHODS

Study Design

The Institutional Animal Care Committee at the University of Alberta approved the experimental protocol. A total of 10 Landrance pigs were used for this experiment.

Donor Liver Procurement

All pigs were premedicated with Atropine (0.05 mg/kg) (Rafter 8, Calgary, Canada) and Ketamine (20 mg/kg) (Bimeda, Cambridge, Canada), after which orotracheal intubation was established, and general anesthesia was maintained with 2% isofluorane (Fresenius Kabi Canada Ltd, Richmond Hill, Canada). A midline laparotomy was performed, and livers were retrieved using a standard technique.[26] All livers were dissected until they were only connected by vascular elements.

TABLE 1.

Selected normothermic ex situ liver perfusion studies

Study	Year	Study type/device	Species	Perfusion temperature, °C	Perfusate composition	Hemoglobin concentration
Nasralla et al.[5]	2018	Clinical transplant (OrganOx *metra*)	Human	37	500 mL Gelofusine +3 units PRBC	Not reported
Watson et al.[4]	2017	Clinical transplant (liver assist)	Human	37	1 L STEEN or Gelofusine +3 units PRBC	Median, 6.1 (5.1-7.4) g/dL
Angelico et al.[14]	2016	Clinical transplant (OrganOx *metra*)	Human	37	1 L "colloid" +3 units PRBC	Not reported
Mergental et al.[3]	2016	Clinical transplant (OrganOx *metra* and liver assist)	Human	37	1000 mL 5% human albumin +3 units PRBC	Not reported
Bral et al.[12]	2016	Clinical transplant (OrganOx *metra*)	Human	37	500 mL Gelofusine +3 units PRBC	104 ± 18 (g/L)
Selzner et al.[11]	2016	Clinical transplant (OrganOx *metra*)	Human	37	500 mL STEEN +3 units PRBC	Not reported
Ravikumar et al.[6]	2016	Clinical transplant (OrganOx *metra*)	Human	37	500 mL Gelofusine +3 units PRBC	Not reported
Watson et al.[15]	2016	Clinical transplant (liver assist)	Human	37	'Erythrocyte-based fluid'	Not reported
Perera et al.[16]	2016	Clinical transplant (liver assist)	Human	37	'Third-party red cell based fluid'	Not reported
Vogel et al.[17]	2017	Experimental perfusion	Porcine	37	1.5 L of pig blood	Not reported
Vogel et al.[18]	2016	Experimental perfusion	Human	37	500 mL Sterofundin +3-4 units PRBC	Not reported
Banan et al.[19]	2015	Experimental perfusion	Porcine	38	1.5 L saline + autologous blood (volume not reported)	Hematocrit 15 to 20
Nassar et al.[20]	2014	Experimental perfusion	Porcine	38	2.5 L heterologous blood + acellular solution combinations ± PRBC	Not reported
Op den Dries et al.[21]	2013	Experimental perfusion	Human	37	750 mL 'Red blood cell concentrate' + 900 mL FFP + 100 mL human albumin	4.7 ± 0.1 mmol/L
Boehnert et al.[22]	2013	Experimental transplant	Porcine	38	3 L STEEN solution	Acellular
Xu et al.[23]	2011	Experimental perfusion	Porcine	39	1.5 L autologous blood +0.5 L sterile porcine plasma	Not reported
Fondevila et al.[24]	2011	Experimental transplant	Porcine	38	Autologous blood (volume not reported)	Not reported
Brockmann et al.[25]	2009	Experimental transplant	Porcine	38	1.5 L autologous blood	Not reported
Butler et al.[26]	2002	Experimental perfusion	Porcine	39	1.5 L of heterologous blood	Not reported
Schoen et al.[27]	2001	Experimental transplant	Porcine	38	2 L whole blood +1 L "balanced electrolyte" solution	Not reported

A median sternotomy was performed, and a 2-stage venous cannula was inserted into the right atrium. Intravenous heparin (Fresenius Kabi Canada Ltd), was administered (30000 units), and the infrarenal aorta was cannulated with a 20 French cannula. The animals were then exsanguinated via the cannula in the right atrium, and the blood was collected to prime the perfusion circuit. Aortic cross-clamp was established. The suprahepatic vena cava was divided near to the heart for venous venting, and the abdominal organs were then flushed with 2 L of cold (4°C) histidine-tryptophan-ketoglutarate (Custodiol HTK, Methapharm Inc., Brantford, ON, Canada) solution.

Ex Situ Perfusion Circuit Design

The locally designed experimental ex situ perfusion circuit was assembled using the following components: a Medtronic Affinity NT oxygenator, 2 BPX-80 Bi-Medicus centrifugal pumps (Medtronic, Minneapolis, MN), and a leukocyte arterial blood filter (LeukoGuard LG; PALL Medical, Port Washington, NY) (Figure 1). The centrifugal pumps were computer controlled to maintain the desired hepatic artery and portal vein pressures. Oxygen and carbon dioxide flows were titrated through the membrane oxygenator to maintain a partial pressure of arterial oxygen between 130 and 200 mm Hg, and a partial pressure of carbon dioxide of 35 to 45 mm Hg.

A sufficient quantity of whole blood was added to Krebs-Henseleit with albumin solution (glucose, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, sodium phosphate monobasic, 8% bovine serum albumin) to achieve an ex situ circuit perfusate with a target mean hemoglobin level of 50 g/L. The circuit was primed with bolus additives including cefuroxime 750 mg (SteriMax Inc., Oakville, Canada), methylprednisolone sodium succinate 500 mg (Pfizer Canada Inc., Kingston, Canada), and sodium heparin 10000 U (Pharmaceutical Partners Canada, Richmond Hill, Canada). Sodium bicarbonate 8.4% (Hospira, Montreal, Canada) was added as needed to maintain pH between 7.35 and 7.45. Continuous infusions were established of 2 IU/h of regular insulin (Eli Lilly Canada Inc., Toronto, Canada), and 1000 units/h of sodium heparin.

Ex Situ Liver Perfusion

After procurement, livers were flushed with 1 L of 0.9% normal saline. The livers were then connected and perfused on our ex situ liver perfusion circuit, at a temperature of 39°C. Livers were perfused through both the hepatic artery and portal vein. Both of the cannulated vessels were under automated computer pressure control, with the hepatic artery perfused at a setpoint of 70 mm Hg and the portal vein perfused at a setpoint of 2 mm Hg. All perfusions were initially commenced at a target mean hemoglobin level of 50 g/L and allowed to proceed for 2 hours. We then performed hourly serial dilutions of the perfusate with additional Krebs-Henseleit with albumin solution to systematically perfuse the livers at decreasing levels of hemoglobin (dilution 1 mean hemoglobin, 30 g/L; dilution 2 mean hemoglobin, 20 g/L; dilution 3 mean

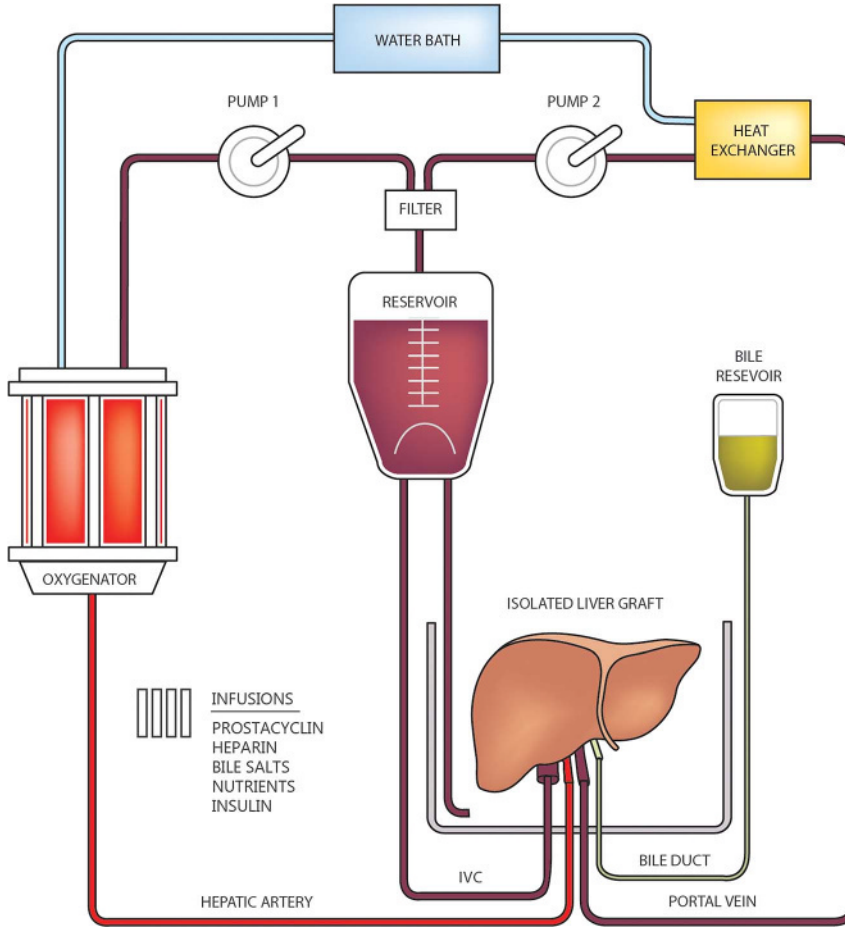

FIGURE 1. Schematic diagram of the experimental ex situ circuit.

hemoglobin, 10 g/L; dilution 4 mean hemoglobin, 7.5 g/L; dilution 5 mean hemoglobin level, 5 g/L). Each dilution interval was allowed to proceed for 1 hour, at which point perfusate samples were withdrawn, centrifuged, and supernatant was stored at −80°C until biochemical analysis was performed. Blood gasses were also analyzed on site. At the end of each perfusion run (mean target hemoglobin level 5 g/L), tissue samples were taken for histological analysis. One perfused liver was used exclusively for further histological analysis, with samples taken at the end of each dilution point.

In the control group, perfusions were established as above and were performed for durations of 24 hours at target hemoglobin levels of 30 and 20 g/L. Perfusate samples were collected, centrifuged, and supernatant was stored at −80°C until biochemical analysis was performed. Blood gasses were also analyzed on site (Figures 2A and B).

Perfusate Composition Analysis

Hemoglobin, electrolyte, pH, total bilirubin, lactate, and partial pressures of oxygen and carbon dioxide were measured using the ABL Flex Analyzer (Radiometer Medical ApS, Bronshoj, Denmark). Perfusate samples were obtained from the hepatic artery circuit at the end of each dilution perfusion and sent for the analysis of aspartate transaminase (AST), alanine aminotransferase (ALT), total bilirubin, lactate dehydrogenase (LD), and lactate using a BeckmanCoulter Unicel Dxc800 Syncron (Brea, CA).

Hepatic Oxygen Consumption and Vascular Resistance

Liver graft oxygen consumption was calculated using the Fick equation, based on arterial and venous blood gases, and compared between different hemoglobin levels.[26] Hepatic artery and portal vein vascular resistance was also compared between each successive hemoglobin level. Vessel resistances were calculated by dividing the pressure by the flow indexed to 100 g of liver tissue.

Histology

Livers were fixed in 10% formalin. Liver tissue samples were analyzed at the end of each hemoglobin dilution, embedded in paraffin, stained with hematoxylin and eosin, and examined in a blinded fashion by an expert pathologist who assigned a semiquantitative score to evaluate for hepatocyte injury and bile sequestration. Biopsy tissue was examined for necrosis (0, absent; 1, pericentral; 2, zones 2 and 3; 3, panlobular); hemorrhage (score: 0, absent; 1, focal; 2, zonal; 3, panlobular), cholestasis (score: 0, absent; 1, present); and sinusoidal dilatation (score: 0, none; 1, mild; 2, moderate; 3, severe), as previously reported.[25]

Statistical Analysis

Data are represented as means ± standard error of the means (SEM). Normally distributed continuous variables were compared using a 1-way analysis of variance with Tukey multiple comparisons. Overall comparison between hemoglobin groups was performed with a 95% confidence interval. A P value less than 0.05 was considered statistically significant and all the analysis was performed using Graphpad Prism (GraphPad Software Inc., La Jolla, CA).

RESULTS

Livers were subjected to comparable periods of cold ischemia before ex situ liver perfusion was initiated (34 ± 3 min). Ex situ liver perfusions were established as described, with a mean initial hemoglobin of 50 ± 1.9 g/L. After 2 hours of perfusion, the serial perfusate dilutions were commenced (dilution 1 mean hemoglobin, 31 ± 1.2 g/L; dilution 2 mean hemoglobin, 21 ± 1.0 g/L; dilution 3 mean hemoglobin, 11 ± 0.6 g/L; dilution 4 mean hemoglobin, 7 ± 0.5 g/L; dilution 5 mean hemoglobin level, 5 ± 0.5 g/L).

The perfusate levels of liver transaminases, LD, and lactate were evaluated and compared at each hemoglobin level. Hepatic transaminases (AST and ALT) rose progressively with each hemoglobin dilution, higher at level of 5 ± 0.46 g/L, as compared with 50 ± 1.9 g/L, although this was not significant (P = 0.09, P = 0.06, respectively) (Figures 3A and B). With increasing serial hemoglobin dilution, higher levels of lactate were observed within the perfusate, first noticed at a hemoglobin level of 21 ± 1.0 g/L (0.4 ± 0.19 mM). This accumulation first becomes significant at a hemoglobin level of 11 ± 0.6 g/L (2.36 ± 0.87 mM) and continues throughout lower hemoglobin levels (Figure 3C). In parallel, levels of LD also increased sequentially (P < 0.001), more noticeably at 21 ± 1.0 g/L (756 ± 294 U/L) of hemoglobin, reaching significance in hemoglobin dilutions less than 11 ± 0.6 g/L (1185 ± 486 U/L) (Figure 3D).

Vascular parameters also demonstrated changes over different hemoglobin levels. Hepatic oxygen consumption

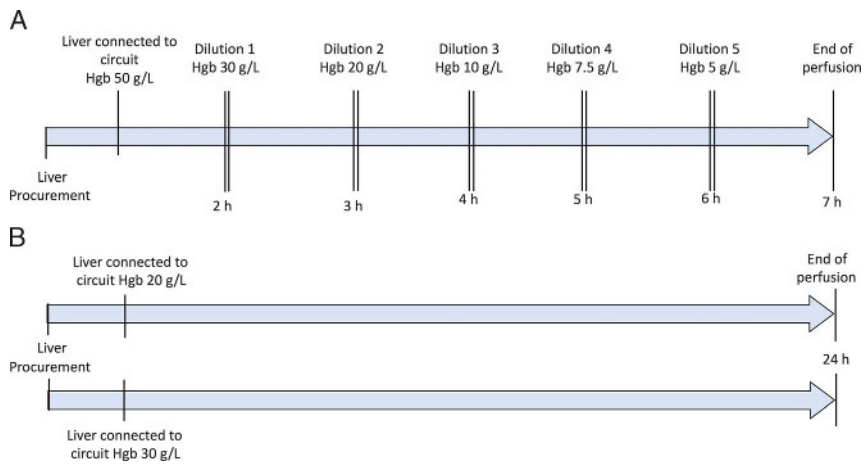

FIGURE 2. Flow diagrams of the experimental ex situ design. *A*, Schematic diagram of the ex situ liver perfusion treatment group. *B*, Schematic diagram of control livers perfused for 24 hours at a constant hemoglobin level.

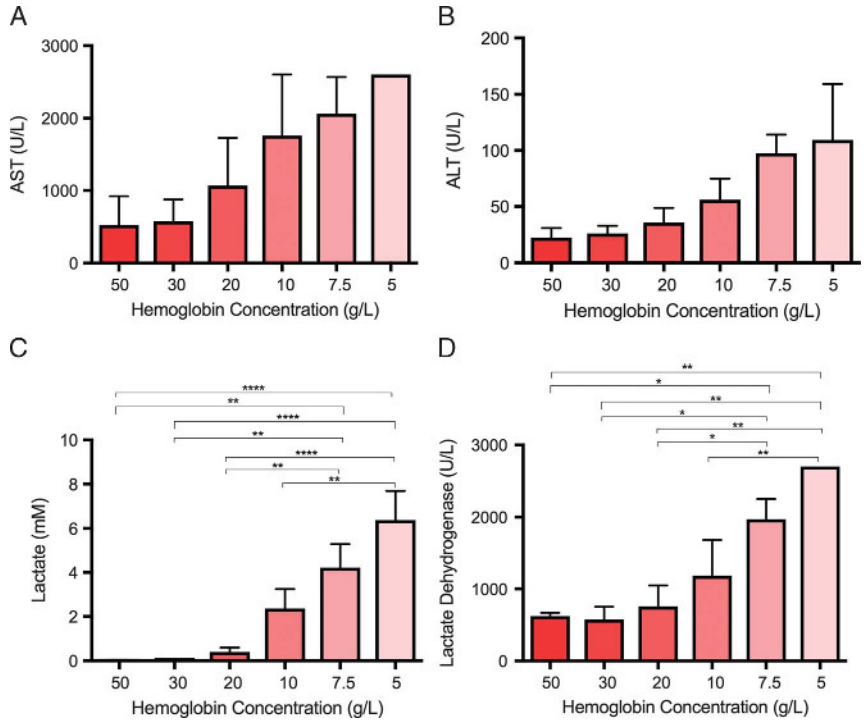

FIGURE 3. Ex situ circulating perfusate liver biochemistry after successive perfusate dilutions during NMP. *A*, Circulating AST perfusate levels at each respective hemoglobin level ($P = 0.09$). *B*, Circulating ALT perfusate levels during NMP, ($P = 0.06$). *C*, Ex situ circulating perfusate lactate levels at each hemoglobin level ($P < 0.001$). *D*, LD levels at each hemoglobin level ($P < 0.001$). Data points show means and SEM, 95% confidence interval. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

decreased sequentially throughout hemoglobin dilutions, with significance ($P < 0.0001$) observed at hemoglobin levels diluted below 50 ± 1.9 g/L (3.14 ± 0.4 mL O_2/min/100 g) (Figure 4A). As expected, hepatic oxygen delivery was also significantly reduced as the concentration of hemoglobin decreased ($P < 0.0001$), with significance noted at levels below 50 ± 1.9 g/L (9.52 ± 1.49 mL O_2/min/100 g) (Figure 4B). Hepatic artery resistance remained relatively stable over the dilutions ($P = 0.33$) (Figure 4C). Portal vein resistance did rise when hemoglobin reached 5 ± 0.46 g/L

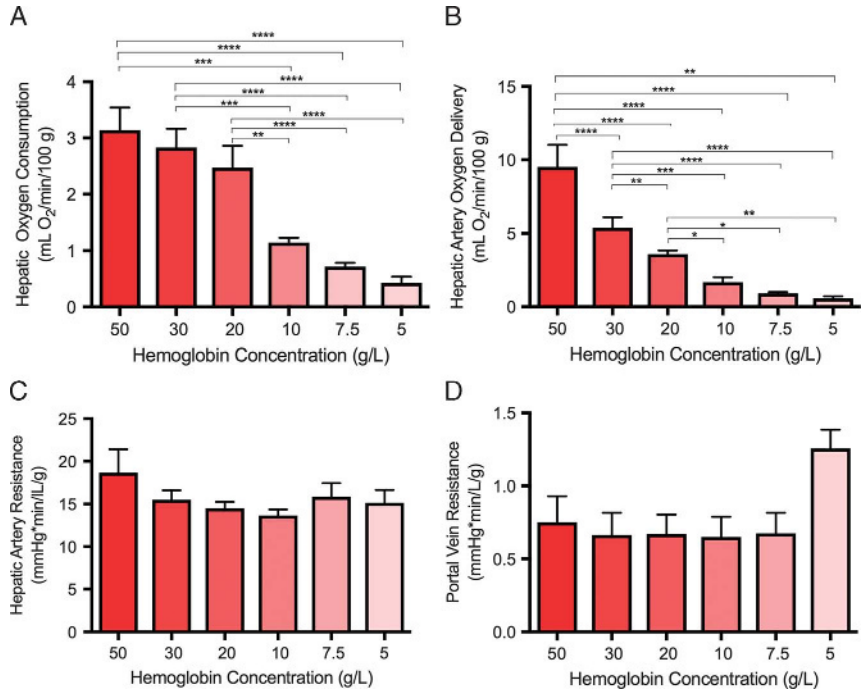

FIGURE 4. Normothermic ex situ liver vascular parameters after successive perfusate dilutions during NMP. *A*, Hepatic oxygen consumption during NMP, at each hemoglobin level group ($P < 0.001$). *B*, Hepatic artery oxygen delivery ($P = 0.001$). *C*, hepatic artery resistance at each hemoglobin level ($P = 0.33$). *D*, Portal vein resistance at each hemoglobin level ($P = 0.15$). Data points show means and SEM, 95% confidence interval. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

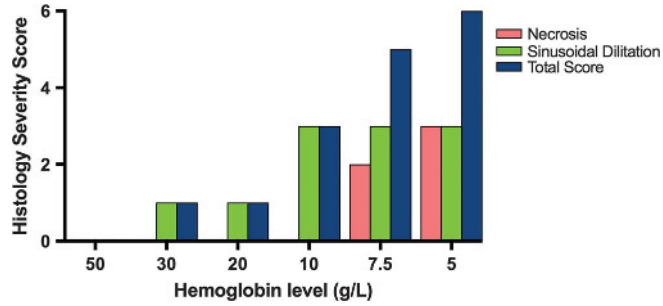

FIGURE 5. Histological scoring of liver biopsy samples after each successive hemoglobin dilution.

(1.25 ± 0.13 mm Hg·min^{-1}·L^{-1}·g^{-1}·), although this was not significant (P = 0.15) (Figure 4D).

Histological analysis revealed that the development of hepatocyte injury was more clearly evident with each successive hemoglobin dilution. Livers perfused at increasingly lower hemoglobin levels demonstrated increased evidence of sinusoidal dilatation and eventual necrosis (Figures 5 and 6).

In the control group, levels of liver transaminases, lactate, and LD were compared over the duration of the perfusions. Hepatic transaminases increased over time, higher in livers perfused at a hemoglobin level of 20 (Figures 7A and B). Higher levels of lactate were observed over time in the perfusate with a hemoglobin level of 20 g/L (Figure 7C). Levels of LD also increased progressively, more so at hemoglobin level of 20 g/L (Figure 7D).

In the control group, histological analysis again confirmed that hepatocyte injury was more evident in livers perfused at the lower hemoglobin level of 20 g/L as compared with 30 g/L, with increasing sinusoidal dilatation at the end of the 24-hour perfusion period (Figures 8A and B).

DISCUSSION

We demonstrate herein the effect of different perfusate hemoglobin concentrations in an experimental porcine normothermic ex situ liver perfusion model. Sequential hemoglobin dilution resulted in progressively higher circulating perfusate levels of markers of liver cellular injury, escalating pathological injury, and decreasing graft metabolic function.

Normothermic ex situ liver perfusion provides the potential to resuscitate marginal donor livers that might otherwise be discarded from the transplant process, and as such, has potential to increase the donor pool. However, evaluation of such livers is imperative before transplantation, to minimize the possibility of primary nonfunction. The assessment of a liver on an ex situ circuit should occur at or near normothermic conditions, and the circuit perfusate requires an oxygen carrier to meet the metabolic demands of the liver.

In the experimental setting, most normothermic ex situ studies have used whole blood, or whole blood with colloid solution.[20,25-27] Recent improved development of alternative hemoglobin-based oxygen carriers has shown safety in NMP, with potential advantages over blood product oxygen carriers.[30] One isolated experimental porcine study successfully perfused livers under normothermic conditions without an oxygen carrier, demonstrating a protective benefit over SCS.[22] Investigators from Cleveland compared STEEN Solution alone or with erythrocytes, versus whole blood. In the whole blood and erythrocyte groups, levels of transaminases were lower, with livers demonstrating better bile production

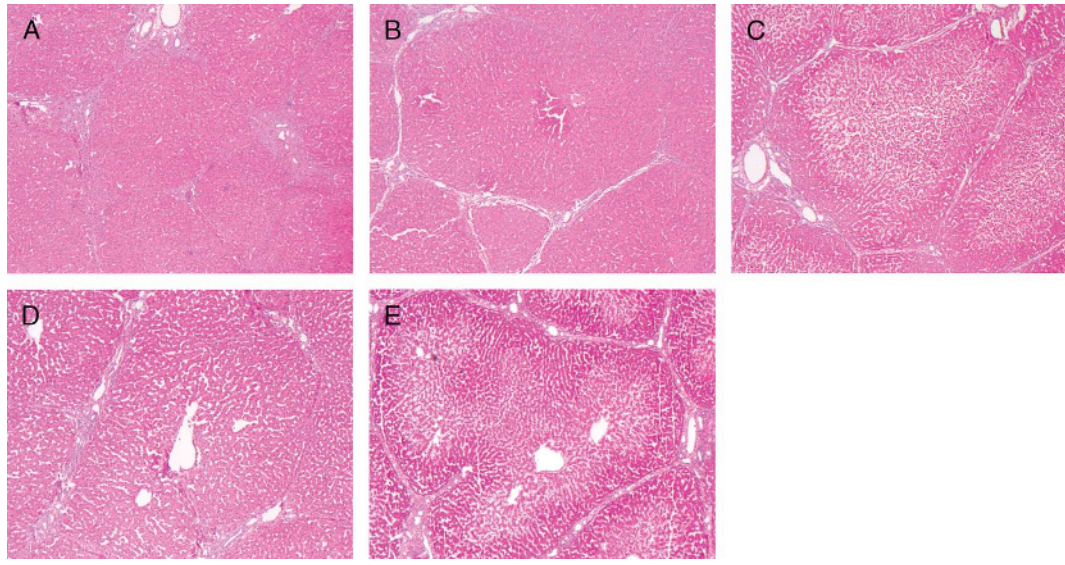

FIGURE 6. Representative histologic sections of liver parenchyma, stained with hematoxylin and eosin, taken after each hemoglobin dilution. *A*, Liver parenchyma at the completion of perfusion with a mean hemoglobin of 50 g/L. *B*, Liver parenchyma at the completion of perfusion with a mean hemoglobin of 31 ± 1.2 g/L. *C*, Liver parenchyma at the completion of perfusion with a mean hemoglobin of 21 ± 1.0 g/L. *D*, Liver parenchyma at the completion of perfusion with a mean hemoglobin of 11 ± 0.6 g/L. *E*, Liver parenchyma at the completion of perfusion with a mean hemoglobin of 7 ± 0.47 g/L.

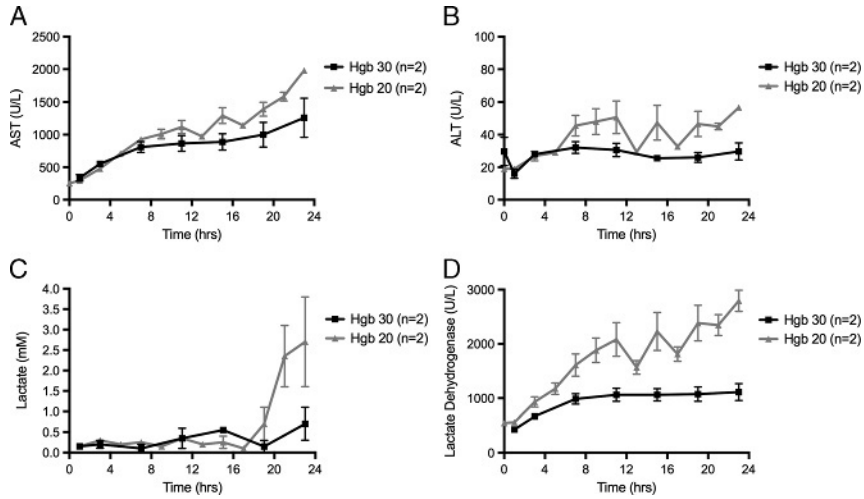

FIGURE 7. Ex situ circulating perfusate biochemistry over 24 hours of NMP. *A*, Circulating AST perfusate levels between groups. *B*, Circulating ALT perfusate levels between control groups. *C*, Ex situ circulating perfusate lactate levels between control groups. *D*, LD perfusate levels over perfusion duration.

and more favorable histology.[31] These findings have also been noted in other organs.[32]

All NMP clinical transplant studies have perfused livers with packed red blood cells (PRBC) and either STEEN Solution or Gelofusine.[6,11,12] To date, no study has demonstrated what level of hemoglobin is adequate to maintain the metabolic needs of a working liver under normothermic ex situ conditions (Table 1).

In most ex situ perfusion studies, higher release of liver transaminases during perfusion is deemed to be an indicator of increased hepatocellular injury. Guarrera et al[33] determined perfusate levels of AST and ALT to strongly correlate with post transplant liver transaminase levels. Monbaliu et al[34] used AST to determine transplantable grafts during hypothermic machine perfusion. In another study, under normothermic conditions, liver transaminases during perfusion were deemed to be predictive of graft recipient survival in a porcine model.[25] In the treatment group, AST and ALT levels rose with each successive hemoglobin dilution, although this did not reach statistical significance. This may have been a result of accumulation of the liver enzymes in a fixed volume of perfusate over time, however, was also offset by the sequential dilution of the perfusate with fresh colloid. The absolute increase in transaminase levels was not determined, and the resultant rise over time may be in fact an underestimation of the real values. Further, liver transaminases are naturally metabolized by the liver, and rising perfusate transaminase levels may be a result of a metabolically failing graft. The higher transaminase increase in livers perfused at a hemoglobin level of 20 g/L in the control group may be a result of this as well. The significance of liver transaminases in closed circuit remains to be elucidated.

To date a number of groups have proposed that perfusate lactate is one of the more relevant surrogate markers of graft viability on an ex situ circuit.[4,21,35] In the treatment perfusions, lactate levels sequentially elevated with each successive hemoglobin dilution. Under normothermic ex situ conditions, on-circuit lactate clearance is considered an indicator of good graft function.[3] Rising perfusate lactate levels in control livers perfused at a lower hemoglobin level may result from the combination of increased anaerobic metabolism and inferior lactate clearance, both of which may have resulted from progressing metabolic failure.

Lactate dehydrogenase is a well-defined marker of cellular injury and has been reported previously by a number of groups in ex situ perfusion studies.[34,36] Both in the treatment and control groups, livers perfused at lower hemoglobin levels demonstrated higher levels of LD, likely a result of increasing cellular damage. The source of rising perfusate LD is not known, and may have resulted from either increasing liver cellular injury, or alternatively hemolysis. Although hemolysis is a well-known complication of machine perfusion, the higher levels of LD in the control livers perfused at a 20-g/L support the assumption that this was of liver origin.

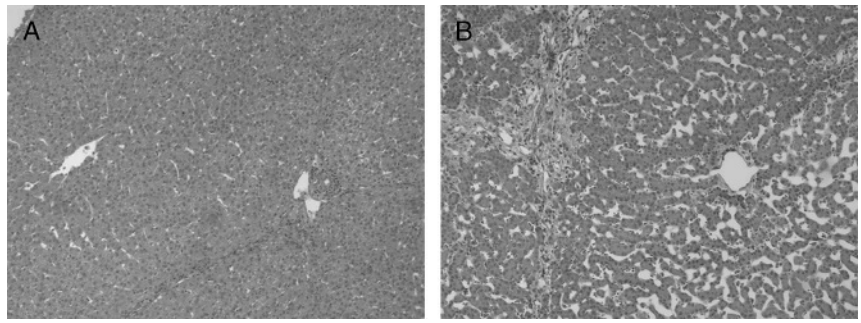

FIGURE 8. Representative histologic sections of liver parenchyma, stained with hematoxylin and eosin, taken from control livers at the end of 24 hours of perfusion. *A*, Liver parenchyma at the completion of perfusion with a hemoglobin level of 30 g/L. *B*, Liver parenchyma at the completion of perfusion with a hemoglobin level of 20 g/L.

With progressive hemoglobin dilution, there was significantly lower liver oxygen consumption at lower hemoglobin levels which paralleled oxygen delivery. Published data on oxygen consumption during ex situ perfusion demonstrate conflicting findings, with most studies reporting higher oxygen consumption as associated with better outcomes.[22] Utilizing NMP, Boehnert et al[22] noted that deceased circulatory death grafts that were preserved using SCS for longer periods of time demonstrated a rapid drop in oxygen consumption, as compared with livers preserved by NMP, indicative of deteriorating metabolic activity.

Vascular resistance has previously been described as a possible marker for graft viability.[25] We found that hepatic artery resistance did not change significantly at different hemoglobin levels. In our study, portal vein resistance did not increase significantly until the lowest hemoglobin level, possibly indicating that this is a late indicator of a failing liver on NMP.

Based on these findings, it seems that a hemoglobin concentration of 31 ± 1.2 g/L is sufficient to preserve liver metabolism, whereas at a hemoglobin level of 21 ± 1.0 g/L, the oxygen supply to the organ barely meets the demand, with a resultant rise in lactate levels. We surmise that perfusions performed at a hemoglobin level higher than 31 ± 1.2 g/L is not detrimental to liver metabolism.

The development of liver parenchymal injury with each successive hemoglobin dilution, as evidenced by histology, may possibly be explained by the generation of reactive oxygen species, leading to endothelial cell damage and increased microvascular permeability. As the perfusate was progressively more dilute, the buffering and antioxidant capacity of whole blood was likely increasingly overwhelmed. Liver damage is notably advanced on histology at hemoglobin levels below 20 g/L, suggesting that this threshold is too low to perform NMP safely. Using our circuit and perfusate, we sought to demonstrate the extreme physiological parameters that a liver will tolerate. In a large mammal experimental model, where multiple organs are often procured at the same time, this may provide a parameter for more optimal distribution of blood product between circuits.

There are a number of important limitations to our study. The serial dilution of perfusate within the experimental group makes it difficult to know with certainty whether observed effects are truly due to the hemoglobin dilution or due to the state of the liver at the start of the dilution, or the damaged state of the liver in general. The rationale behind the methodology was that in NMP, if a liver is functioning well, observed changes in perfusate biochemistry, such as decreasing lactate, occur in short periods.[3,4,16]

Each hemoglobin level dilution was allowed to proceed for only 1 hour, and had this period been extended, some observations may have become more pronounced, or alternatively, the livers may have shown signs of failure at higher hemoglobin levels. The effect of the physiologic solution in contributing to progressive graft damage is unknown. Further only machine perfusion was performed, without a transplant recovery model, which likely would have strengthened the observations. Our conclusions are restricted to this porcine large animal model and the type of ex situ normothermic perfusion circuit and additives chosen. Altering any of these conditions could potentially alter the translatability of the findings.

Despite recent progress in developing alternative oxygen carriers, to date, all clinical NMP transplant studies have been performed with human PRBCs as the oxygen carrier. Blood products continue to be a universally scarce resource, and the implications of performing MP at lower hemoglobin levels may result in more rational resource utilization, simplified logistics, and cost savings.

Based on this study, we conclude that under normothermic ex situ perfusion conditions, a hemoglobin concentration of between 30 and 20 g/L should be maintained to assure optimal graft function. A value higher than this is not harmful and may be beneficial. Our experimental findings here suggest that this target may provide a reasonable reserve, and that the total ex situ NMP hemoglobin concentration does not need to be in the normal physiological range. Such a metabolically intact liver is necessary if any potential viability measures are to give some indication of posttransplant function.

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