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Continuous Renal Replacement Therapy During Long-term Normothermic Machine Perfusion of Human Donor Livers for up to 7 D

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Background. Normothermic machine perfusion (NMP) is used to preserve and test donor livers before transplantation. During NMP, the liver is metabolically active and produces waste products, which are released into the perfusate. In this study, we describe our simplified and inexpensive setup that integrates continuous renal replacement therapy (CRRT) with NMP for up to 7 d. We also investigated if the ultrafiltrate could be used for monitoring perfusate concentrations of small molecules such as glucose and lactate. **Methods.** Perfusate composition (urea, osmolarity, sodium, potassium, chloride, calcium, magnesium, phosphate, glucose, and lactate) was analyzed from 56 human NMP procedures without CRRT. Next, in 6 discarded human donor livers, CRRT was performed during NMP by integrating a small dialysis filter (0.2 m^2) into the circuit to achieve continuous ultrafiltration combined with continuous fluid substitution for up to 7 d. **Results.** Within a few hours of NMP without CRRT, a linear increase in osmolarity and concentrations of urea and phosphate to supraphysiological levels was observed. After integration of CRRT into the NMP circuit, the composition of the perfusate was corrected to physiological values within 12 h, and this homeostasis was maintained during NMP for up to 7 d. Glucose and lactate levels, as measured in the CRRT ultrafiltrate, were strongly correlated with perfusate levels ($r = 0.997$, $P < 0.001$ and $r = 0.999$, $P < 0.001$, respectively). **Conclusions.** The integration of CRRT into the NMP system corrected the composition of the perfusate to near-physiological values, which could be maintained for up to 7 d. The ultrafiltrate can serve as an alternative to the perfusate to monitor concentrations of small molecules without potentially compromising sterility.

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Normothermic machine perfusion (NMP) is a method to preserve or test (extended criteria) donor livers before transplantation.[1] During NMP, the liver is perfused at a temperature between 35 and 37 °C, which results in a metabolically active liver. This provides the opportunity to test the liver function before transplantation. However, a metabolically active liver also produces waste products that accumulate in the perfusion solution (perfusate) during NMP.[2] This accumulation of (waste) products, such as urea and sodium, may potentially harm the liver. Increased osmolarity, for example, can induce hepatocyte shrinking, followed by the production of reactive oxygen species and, ultimately, cell death and potential graft loss.[3-5]

To maintain a perfusate composition within ranges similar to physiological reference values in human circulation, a form of continuous renal replacement therapy (CRRT) can be used. This has already been explored in previous experimental NMP settings using rat, porcine, and human livers, wherein the perfusate maintained a stable pH, physiological electrolyte concentrations, and accumulation of harmful waste products was prevented.[6-9] However, none of the currently available

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commercial liver machine perfusion devices have integrated CRRT. The reason behind this might be the potentially high costs with conventional dialysis systems (up to USD $20,000–30,000)[10] in combination with acceptable outcomes after short-term (<24 h) NMP without CRRT or any other methods of purification of the perfusate.[11-13] Nevertheless, with the extension of perfusion times from 24 h up to multiple days, the addition of CRRT becomes essential to maintain a physiological environment to protect the liver against self-intoxication.[9,14]

Published articles on long-term NMP using a form of renal replacement are scarce.[9,15] These publications primarily center around different aspects of NMP and may not provide readily reproducible setups for other institutions seeking to initiate long-term NMP. To address this, we aimed to elucidate a CRRT setup that does not rely on a costly, conventional dialysis system. During long-term NMP, we incorporated a small dialysis filter, specifically a pediatric hemoconcentrator commonly used in cardiothoracic surgery to counteract hemodilution. The addition of this filter facilitated fluid extraction (ultrafiltration), including the removal of accumulated waste products and various small molecules during the long-term NMP process. Simultaneously, the ultrafiltrate volume is continuously replaced with off-the-shelf substitution solutions commonly used in continuous venovenous hemofiltration for critically ill patients.

Additionally, the ultrafiltrate derived from the CRRT system could serve as a convenient and readily accessible sample source for repeatedly measuring certain low molecular weight perfusate components without potentially compromising sterility.[14,16]

In this study, we retrospectively analyzed a cohort of short-term clinical liver NMP procedures to establish the potential need for CRRT and to identify a time window for when CRRT might be beneficial during NMP. We subsequently investigated the addition of a reproducible CRRT setup in a prospective cohort of 6 discarded human livers that underwent long-term NMP for up to 7 d. We analyzed and compared time courses of urea, osmolarity, and the major electrolytes. Furthermore, we investigated if the ultrafiltrate can be used as an alternative source of circulating glucose and lactate measurements.

MATERIALS AND METHODS

Study Group

To investigate the need for CRRT during NMP, we retrospectively evaluated clinical liver NMP procedures performed in our hospital between January 2019 and November 2022. The selection and procurement procedure of these donor livers is described elsewhere.[12] In addition, 6 human donor livers that were declined for transplantation and offered for research were studied (**Table 1**). These livers were subjected to NMP for up to 7 d, using a modified NMP circuit with integrated CRRT. Informed consent for research was obtained from the donor's relatives by the organ donation coordinators. For this study, approval from the ethics board was not indicated.

Machine Perfusion

For both short-term clinical NMP and for long-term research NMP, a Liver Assist device (XVIVO, Groningen, the Netherlands) was used. The procedure for the clinical NMP is described elsewhere.[12] The long-term research NMP procedure was based on clinical procedures. In brief, before NMP, at least 1-h dual hypothermic oxygenated perfusion (around 10 °C, portal vein pressure ≤5 mm Hg and hepatic artery pressure ≤25 mm Hg, and oxygenated with 1 L/min 100% O_2) was performed, using Belzer MPS machine perfusion solution (Carnamedica, Warsaw, Poland) to protect the liver against ischemia–reperfusion injury at the start of NMP. After this, the liver was briefly disconnected from the machine to switch Belzer MPS for a perfusate containing red blood cells, similar to the perfusate used for clinical NMP procedures (**Table S1, SDC,** http://links.lww.com/TXD/A603). After the perfusate change and subsequent priming of the perfusion device, the liver was reconnected for a 1-h controlled oxygenated rewarming (gradual temperature increase from 20 °C to 37 °C, with a maximum portal vein pressure ≤11 mm Hg and hepatic artery pressure ≤70 mmHg, oxygenated with an air/O_2 mixture), followed by NMP at 37 °C thereafter. During controlled oxygenated rewarming and NMP, some electrolytes were administrated to aim for specific (near physiological) values of sodium (135–145 mmol/L), potassium (3.5–5.0 mmol/L), free ionized calcium (>0.7 mmol/L), and chloride (>90 mmol/L).

TABLE 1.

Donor characteristics

	Liver 1	Liver 2	Liver 3	Liver 4	Liver 5	Liver 6
Donor age, y	74	54	51	25	60	64
Donor gender	Female	Male	Female	Female	Male	Female
Donor BMI, kg/m^2	34	34	35	32	27	51
Cause of death	Cardiac arrest	Trauma	CVA	Suicide	Trauma	Cardiac arrest
Donor type	DCD	DCD	DBD	DBD	DCD	DCD
Reason for declining	Age, nationwide no NMP capacity	Macroscopic steatosis and laceration	Macroscopic steatosis and laceration	↑ PT and fever	History of carcinoma	Macroscopic steatosis
Functional WIT, min	26	43	0	0	29	31
Last sodium, mmol/L	142	144	150	144	150	144
Last AST, U/L	39	15	136	102	27	45
Last ALT, U/L	141	22	190	134	25	78
Last GGT, U/L	129	263	75	155	63	110
Last AP, U/L	77	43	66	113	72	91
Last bilirubin, μmol/L	8	6	5	9	7	6
Static CIT, min	442	359	745	310	198	292

ALT, alanine transaminase; AP, alkaline phosphatase; AST, aspartate transaminase; BMI, body mass index; CIT, cold ischemia time; DBD, donation after brain dead; DCD, donation after circulatory death; GGT, gamma-glutamyltransferase; NMP, normothermic machine perfusion; PT, prothrombin time; WIT warm ischemia time.

Setup of CRRT

For CRRT we used a dialysis filter that can filtrate small molecules, such as urea, effectively from the perfusate by means of transmembrane pressure, while retaining larger molecules, such as hemoglobin and albumin. For this, we selected a pediatric hemoconcentrator filter (BC 20 plus, Maquet, Getinge Netherlands B.V., The Netherlands), which contained a polyarylsulfone membrane with a surface of 0.2 m^2 and a priming volume of 17 mL. It was connected to the arterial circuit of the liver perfusion device, as depicted in **Figure 1**. During the 7 d of NMP, the filter was not replaced.

The perfusate inflow to the filter was positioned after the hepatic artery pump to make use of the highest pressure in the system and to avoid the addition of an extra pump to minimize hemolysis.[14] The perfusate outflow of the filter returned to the venous return line from the organ chamber. At this site

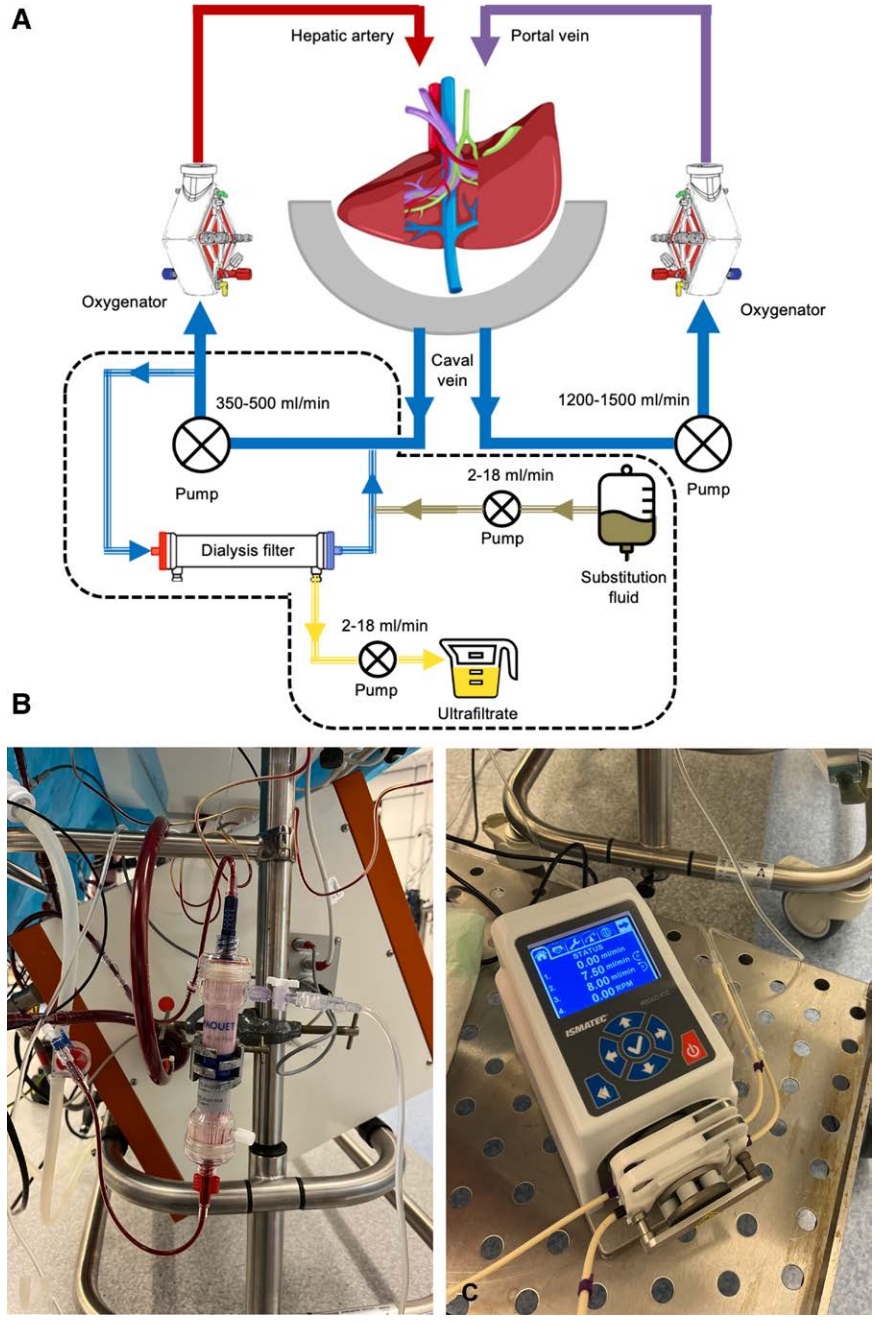

FIGURE 1. A, Setup of the CRRT by addition of a pediatric dialysis filter into the existing Liver Assist disposable set (XVIVO, Groningen, The Netherlands) for liver machine perfusion. The black interrupted line surrounds the added circuit for CRRT to the existing perfusion circuit. The perfusate inflow to the dialysis filter (B) was positioned after the arterial pump to benefit from the highest pressure in the system and to avoid the addition of an extra pump to minimize hemolysis. Perfusate outflow from the dialysis filter was returned to the outflow line coming from the liver reservoir to avoid shunting of hypoxic perfusate. Drainage of ultrafiltrate from the dialysis filter was facilitated by a roller pump (C). The roller pump speed was manually adjusted on the basis of the osmolarity level of the perfusate, which is mainly affected by the sodium, glucose, and urea levels. The substitution fluid inflow was positioned after the dialysis filter to obtain an ultrafiltrate composition that can be used for analyses of glucose and lactate values in the ultrafiltrate. The substitution fluid was connected to a separate roller pump. The speed of the substitution fluid infusion was matched to the ultrafiltrate flow to maintain a stable perfusate hematocrit. CRRT, continuous renal replacement therapy.

of the circuit, the perfusion fluid pressure is the lowest, resulting in a maximum pressure gradient. Also, because the extra circuit is before the oxygenator, there will be no shunt, and all perfusate going to the hepatic artery will be oxygenated.

The tubing of the ultrafiltrate outflow was connected to a roller pump (Reglo ICC, Ismatec, Masterflex, USA) to regulate the ultrafiltrate flow. The flow of the ultrafiltrate was manually adjusted between 2 and 18 mL/min to maintain an osmolarity of the perfusate between 285 and 295 mOsm/L. The substitution fluid infusion line was connected to a separate roller pump set at the same flow rate to mix the substitution fluid with the perfusate, thereby obtaining a zero net balance. Small adjustments in substitution fluid flow were made to compensate for the volume of medication infusion (ie, antibiotics, heparin). Perfusate hematocrit was used as the key parameter to fine-tune substitution fluid administration. Because the CRRT was based on ultrafiltration and not dialysis, no dialysate was used. This allowed us to infuse the substitution fluid into the machine perfusion circuit after the dialysis filter, resulting in an ultrafiltration composition that could be used for analyses of glucose and lactate concentrations in the perfusate.

Several substitution fluids used for clinical CRRT were explored. The primary substitution fluid that we used was Prismasol 2 (Baxter Holding B.V., The Netherlands). Prismasol 2 contains lactate that can be used as a natural energy source in the liver. However, the addition of lactate to the perfusate can also interfere with the lactate clearance capacity of the liver during the first hours of perfusion and interfere with viability criteria for livers perfused with NMP. For this reason, ultrafiltration was only started between 2.5 and 5.5 h after the endogenous lactate was sufficiently cleared <2.5 mmol/L. During perfusion, when there was increased lactate (>2 mmol/L) in the perfusate for several hours, the substitution fluid was changed to biphozyl (Baxter Holding B.V., The Netherlands; liver 1) or phoxilium 1.2 mmol/L phosphate (Baxter Holding B.V., The Netherlands; livers 3 and 4). The compositions of the various commercially available substitution fluids are shown in **Table 2**.

Analyses

During NMP, perfusate samples were analyzed at the start of NMP, and after every 4 to 8 h for urea, osmolarity, and hematocrit, and every 12 h for measurement of sodium, potassium, calcium, chloride, phosphate, and magnesium concentrations, using standard laboratory methods. Also, arterial perfusion fluid and ultrafiltrate samples were collected every 6 h for analysis of lactate and glucose concentrations, using an ABL 90 Flex blood gas meter (Radiometer, Brønhøj, Denmark).

Statistics

Continuous data were presented as medians with interquartile range (IQR). The Pearson correlation test was used with a 95% confidence interval to determine the relationship between the differences in arterial perfusate and ultrafiltrate glucose and lactate measurements. Statistical analyses were performed using Graphpad Prism version 9.5 (Graphpad Software, San Diego, CA). A significance level of 5% (α = 0.05) was used.

RESULTS

Clinical NMP Without CRRT

Between January 2019 and November 2022, 56 consecutive clinical NMP procedures without CRRT were performed. Already within 7 h after initiation of NMP, supraphysiological urea levels (≥30 mmol/L) were reached in the perfusate of some NMP procedures, and levels continued to increase linearly (**Figure 2A**). Assuming a urea distribution volume of 3 L (liver + perfusate), the estimated median urea production rate was 9 mmol/h (30 mmol/L in 10 h × 3L = 9 mmol/h). This increase in urea largely explained the increase in osmolarity to up to 360 mOsm/L (**Figure 2C**), far above the human physiological level (300 mOsm/L). Also, hyperphosphatemia was observed (**Figure 2E**), which stabilized around 5 mmol/L between 3.5 and 8 h of perfusion. No calcium–phosphate depositions were seen in the perfusate. Sodium and potassium levels remained within or near the human physiological range (**Figure 2**G and I); however, chloride, calcium, and magnesium levels were below normal range values (**Figure 2**K, M, and O). Taken together, these results indicate that during short-term NMP (≤10 h) without any CRRT, the perfusate composition changes substantially, with high urea and osmolarity levels and various electrolyte disturbances.

Long-term NMP With Integration of CRRT

Six human donor livers declined for transplantation and offered for research were subjected to long-term NMP. After

TABLE 2.

Composition of different substitution fluids

Composition, mmol/L	Prismasol 2[a]	Biphozyl[a]	Phoxilium[a]
Sodium	140	140	140
Potassium	2	4	4
Calcium	1.75	0	1.25
Bicarbonate	32	22	30
Phosphate	0	1	1.2
Chloride	111.5	122	116
Lactate	3	0	0
Magnesium	0.5	0.75	0.6
Glucose	6.1	0	0
Osmolarity, mOsm/L	297	290	293

[a]All from Baxter Holding B.V., The Netherlands.

several hours (2.5–5.5 h) of NMP, when perfusate lactate levels dropped <2.5 mmol/L, the CRRT system was added to the perfusion circuit. After an initial increase in urea and osmolarity during the first hours of perfusion, the addition of CRRT into the circuit decreased and stabilized the median urea concentration after 12 h of NMP to 7.9 mmol/L (IQR, 5.6–8.6 mmol/L) and osmolarity to 289 mmol/L (IQR, 287–292 mmol/L). During CRRT with a median ultrafiltration

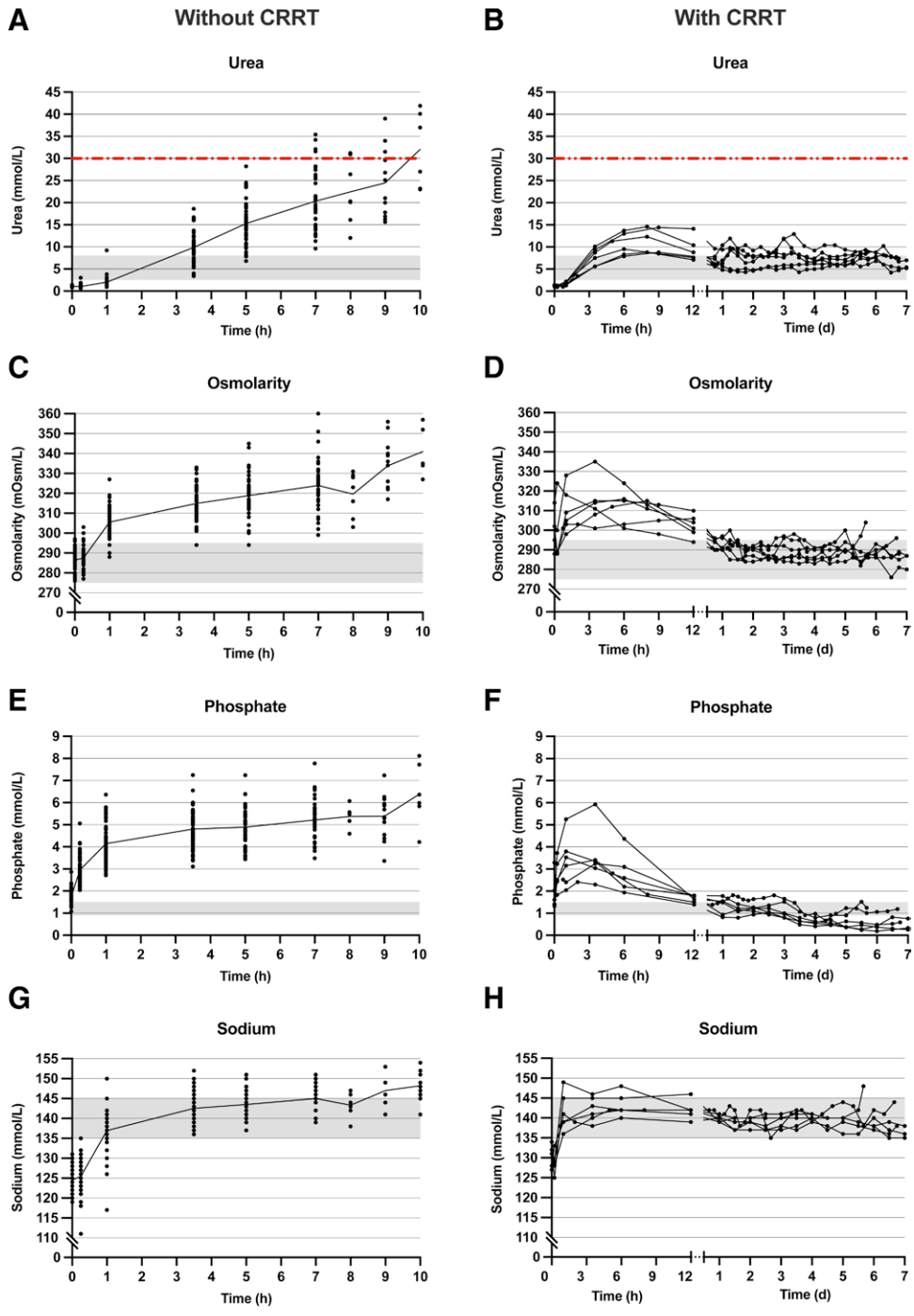

FIGURE 2. Clinical chemistry measurements of urea (A and B), osmolarity (C and D), phosphate (E and F), sodium (G and H), potassium (I and J), chloride (K and L), calcium (M and N), and magnesium (O and P) during clinical, short-term NMP procedures (n = 56 procedures; left panels) and during long-term NMP of 6 human research livers with CRRT incorporated into the perfusion system 2.5 to 5.5 h after initiation of NMP (right panels). The gray field represents the normal plasma values in human adults. The red dotted line within the urea graphs (A and B) indicates a urea level that is considered supraphysiological (30 mmol/L). After 7 h of NMP without CRRT, progressively higher urea levels were reached (A), which did not occur with NMP with CRRT (B). The osmolarity reached supraphysiological values within 1 h after NMP started and continued to increase without CRRT (C) but normalized to physiological values after the incorporation of CRRT (D). The electrolytes were more in physiological range because these were manually administered during the early phase of NMP (G and P). After CRRT was started, the electrolytes were kept in range by the composition of the substitution fluid (H, J, L, N, and P). The vertical red dotted line in the calcium graph (N) is the moment when the substation fluid was changed from prismasol 2 with a calcium concentration of 1.75 mmol/L to biphozyl without calcium, resulting in an almost immediate decrease in calcium level. CRRT, continuous renal replacement therapy; NMP, normothermic machine perfusion.

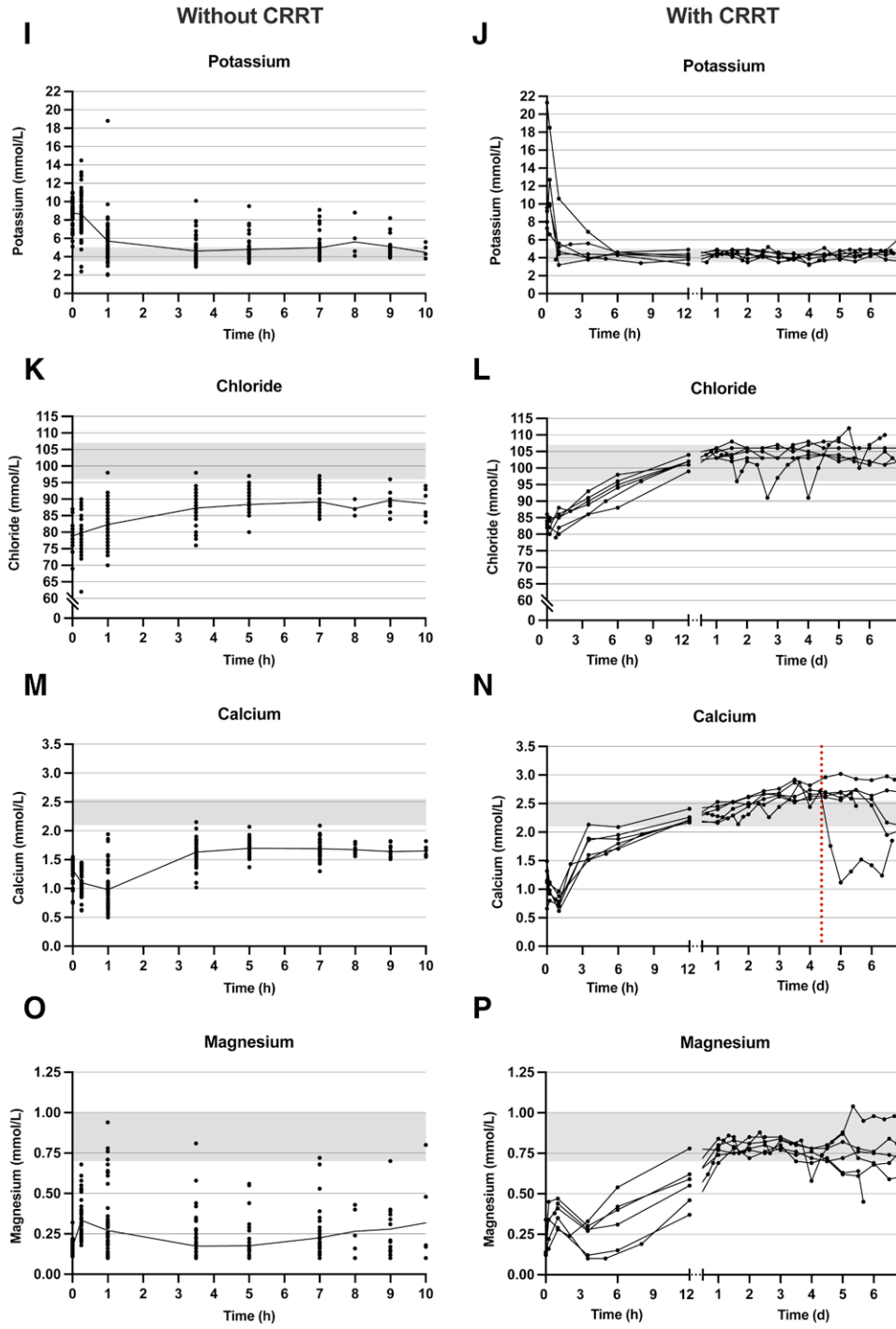

FIGURE 2. Continued

rate of 6.7 mL/min, the urea concentration and osmolality no longer increased to supraphysiological levels and remained within normal ranges (**Figure 2**B and D). Also, electrolytes, such as phosphate, remained within near-physiological values (**Figure 2**F, H, J, L, N, P). The hematocrit values of the 6 livers that served as targets to guide fluid balances are shown in **Figure 3**. No erythrocytes were added to the perfusate after the start of controlled oxygenated rewarming.

The doses of administered electrolytes in the first few hours before starting CRRT were similar to doses administered during the clinical NMPs. Hereafter, the electrolyte concentrations stabilized within normal ranges, and no further adjustments were necessary during the 7-d perfusions. The composition of the substitution fluid is essential to keep the electrolytes stable. For example, for liver 1, the substitution fluid was changed from prismasol 2 (calcium 1.75 mmol/L) to biphozyl (calcium 0 mmol/L) after 4 d (dotted line), resulting in an almost immediate decrease in calcium levels (**Figure 2N**). For the 6 long-term perfused livers, a median of 6.7 mL/min (IQR, 4.7–7.8 mL/min) of ultrafiltrate was removed by the CRRT, which was repleted with 6.2 mL/min (IQR, 4.4–7.6 mL/h) of substitution fluid, in addition to medication infusions. The

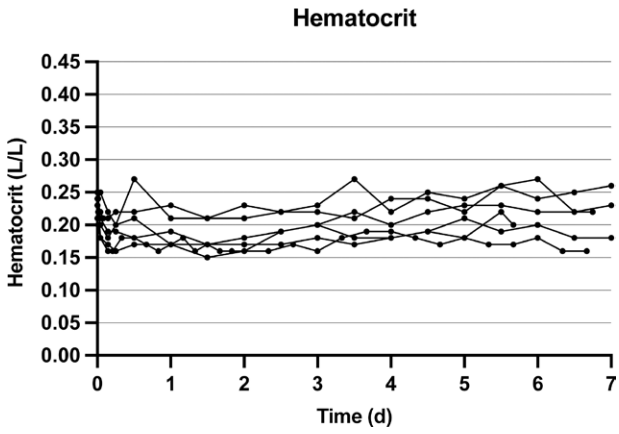

FIGURE 3. Hematocrit measurements during long-term normothermic machine perfusion of 6 human research livers with renal replacement therapy incorporated into the perfusion device. The hematocrit levels were used to guide the overall fluid balance.

adjustments in ultrafiltration for the 6 livers are shown in **Figure 4**. On day 5, liver 2 showed a sudden increase in perfusate lactate levels, possibly due to liver failure.

CRRT and Ultrafiltrate Analyses

Glucose and lactate levels in both the perfusate and ultrafiltrate from CRRT were compared with assessing the feasibility of using the latter as an alternative to repeated sampling from the perfusate. This comparison was conducted across a total of 141 measurements. The correlation between glucose and lactate levels in the perfusate and in the ultrafiltrate was excellent, with correlation coefficients of $r = 0.997$ ($P < 0.001$) and $r = 0.999$ ($P < 0.001$), respectively (**Figure 5**A and B). An analysis of the correlation plot reveals that the majority of lactate levels detected in the ultrafiltrate were higher when compared with those in the perfusate. It is worth noting that the plasma lactate concentration, which serves as the source for the ultrafiltrate, is inherently higher than the lactate concentration in whole blood.[17]

DISCUSSION

Renal support is currently not incorporated in clinically approved commercial liver NMP devices. This study shows that after 7 h of NMP, increasingly high urea levels were observed in the perfusate, producing a supraphysiological osmolarity. The addition of CRRT with a median ultrafiltration rate of 6.7 mL/min to the NMP system normalized these values to (near-) physiological levels. We also observed normalization of electrolyte levels, including sodium, and this homeostasis was maintained for a duration of 7 d.

The composition of the perfusate during clinical liver NMP has largely been overlooked. Thus far, few papers have addressed this topic although the changing composition of the perfusate may potentially be harmful to the liver.[18] Especially in the first few hours of NMP, the liver changes from a low metabolic state to a full metabolic state, including activation of ion channels with changes in perfusate electrolyte composition.[18] For this reason, electrolytes such as potassium, sodium, calcium, and chloride must be administered in the first few hours. These initial shifts in electrolyte composition are inevitable; however, more long-term changes should be minimized during NMP. Supraphysiological levels of phosphate, for instance, can result in the precipitation of calcium–phosphate complexes, leading to deposits in the perfusate and a decrease in levels of free calcium.[19]

Osmolarity shifts that can cause cell death and severe intrahepatic edema in the recipient posttransplantation, potentially resulting in graft loss, which should be avoided.[3-5] Therefore, we also monitored the osmolarity level of the perfusate to assess the effectiveness of CRRT, instead of monitoring only electrolytes as in other studies.[9,15] The osmolarity is primarily influenced by the levels of sodium, glucose, and urea. Because the liver is metabolically active during NMP, urea continues to accumulate even when sodium and glucose are controlled, resulting in a persistent increase in osmolarity.

Another approach to removing the accumulation of (waste) products during long-term NMP is to perform intermittent (partial) exchange of the perfusate.[20] This removes the toxic waste products but also induces fluctuations in the perfusate composition, such as a certain drop in osmolarity. This is why in critically ill patients, CRRT is preferred, to minimize hemodynamic and electrocyte fluctuations.[21] Furthermore, with the substitution of the perfusate, a considerable amount of perfusate is unnecessarily thrown away, often containing valuable and scarce human blood products such as erythrocytes. During our long-term NMP procedures with a dialysis filter, there was no need for the addition of red blood cells throughout the perfusion, and we could keep the hematocrit stable at approximately 0.2 L/L.

When CRRT is included in NMP, such intermittent perfusate changes are not required. CRRT can be complicated and expensive, and in previous long-term NMP studies, the way by which RRT was combined with the system was difficult to elucidate from published material.[9,15] We chose to use a straightforward and relatively inexpensive setup with a small (pediatric) ultrafiltration membrane directly inserted in the arterial segment. The use of 2 separate roller pumps, both set at a similar rate between 2 and 18 mL/min, regulated and balanced the ultrafiltrate and the substitution flows, minimizing undesired fluctuations in the perfusate volume. In intensive care unit patients, the most frequently used methods for CRRT are continuous venovenous hemofiltration, continuous venovenous hemodialysis, or a combination of the previous 2.[22] Continuous venovenous hemofiltration is based on convection, whereas continuous venovenous dialysis is based on diffusion by a countercurrent or cocurrent dialysate flow into the dialysate compartment of the filter. In all cases, the ultrafiltrate is replaced by a substitution fluid.[22] The small dialysis filter we used is officially not designed for CRRT. It is typically used during cardiothoracic surgery in the cardiopulmonary bypass machine to counteract hemodilution by removing excessive fluid.[23] But this filter effectively achieved adequate ultrafiltration of small molecules such as urea and sodium by convection while retaining larger molecules, such as albumin, that did not have to be supplemented.

An additional benefit of the incorporation of CRRT into the NMP circuit is the possibility to (continuously) measure certain perfusate components in the ultrafiltrate as a surrogate for repeated sampling of the perfusate. Frequent perfusate analysis is important for liver viability assessment during NMP; however, sequential measurements during long-term

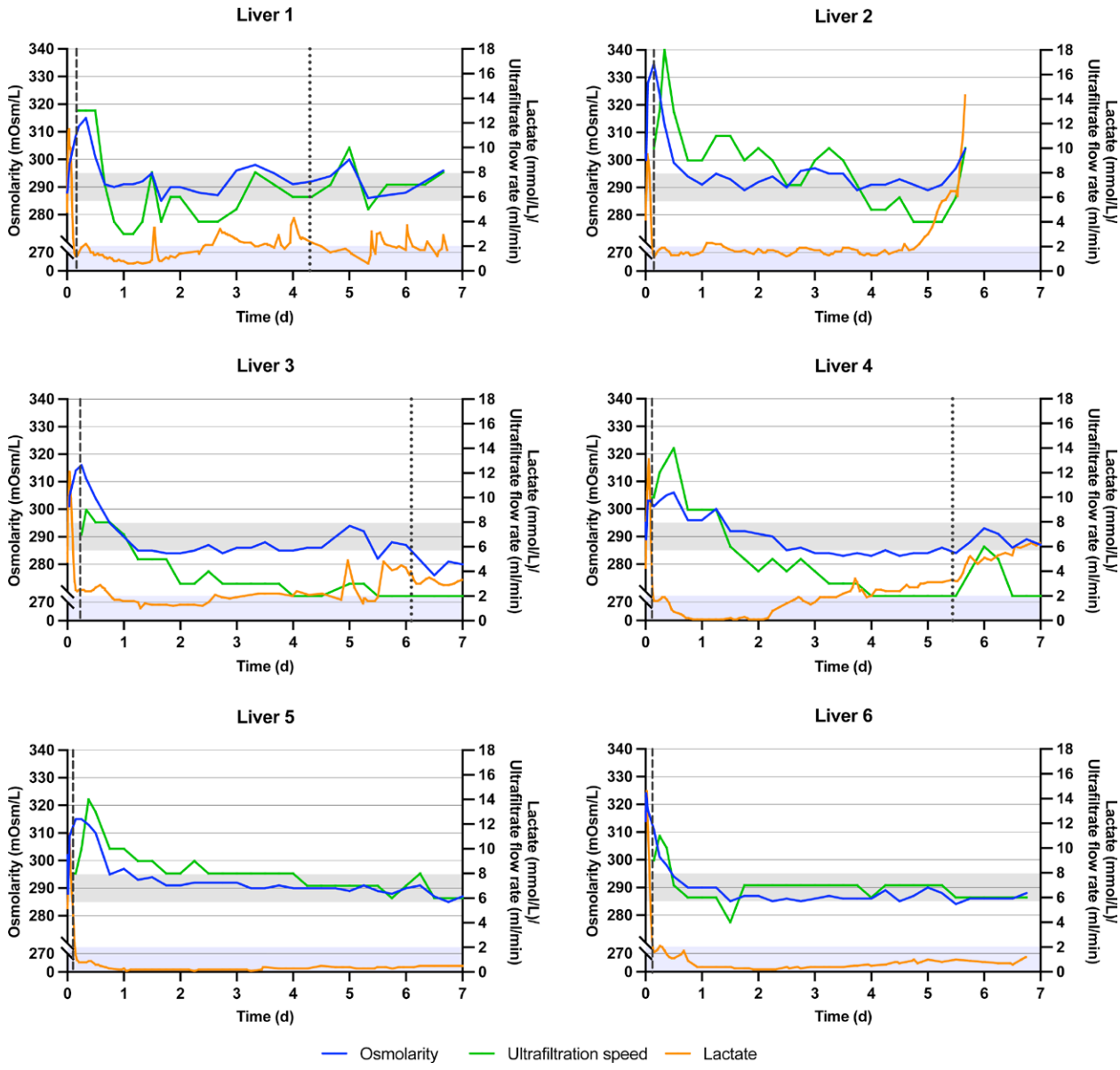

FIGURE 4. Osmolarity of the perfusate (blue line) and ultrafiltrate flow rate (green line) from research livers 1 to 6 to demonstrate the effect of the adjustments made in the ultrafiltrate flow rate on the perfusate's osmolarity by the removal of urea and sodium. The black vertical hatched line indicates when continuous renal replacement therapy ultrafiltration was initiated. The gray field represents the normal plasma osmolarity range in humans. The graphs also include the lactate concentration of the perfusate (orange line). The light-blue field represents the preferable lactate concentration in the perfusate (≤2 mmol/L). When lactate was >2 mmol/L for several hours, the substitution fluid was changed to a fluid without lactate (back dotted lines).

perfusion can increase the risk of contamination and may substantially decrease the total circulating volume of the perfusate.[14,16] Analysis of the ultrafiltrate may, therefore, be an attractive substitute to avoid the need for repeated sample collection from the perfusate. We focused on glucose and lactate because glucose levels need to be maintained in a certain range to prevent excessive glycogen depletion or deposition, and lactate levels can be used to monitor hepatocellular function.[9] That the lactate levels were similar or lower in the perfusate compared with ultrafiltrate (**Figure 5**), is in agreement with what would be expected at the hematocrit values, because intraerythrocyte lactate is lower than plasma lactate.[24]

Although we have only examined 6 discarded human research livers for up to 7 d of NMP, the beneficial effects of the addition of CRRT were comparable and consistent among all livers. We initiated ultrafiltration after reaching the time point (ie, 2.5 h after the start of NMP) used for viability assessment during clinical NMP. In this way, we could use the clinical viability criteria as a baseline for the quality of the hepatocellular and cholangiocellular function. Unfortunately, this artificially creates a peak in lactate and accumulation of (waste) products that are ideally avoided for long-term NMP. For future experiments, an earlier start of ultrafiltration (ie, before the start of NMP) can be anticipated to minimize changes in the perfusate during NMP.

Another limitation of this preclinical study is that these research livers were not subsequently transplanted. Given that long-term NMP is still in its infancy, it is important to note ethical and safety concerns currently prohibit the transplantation of a liver after several days of NMP.[25] Therefore, the

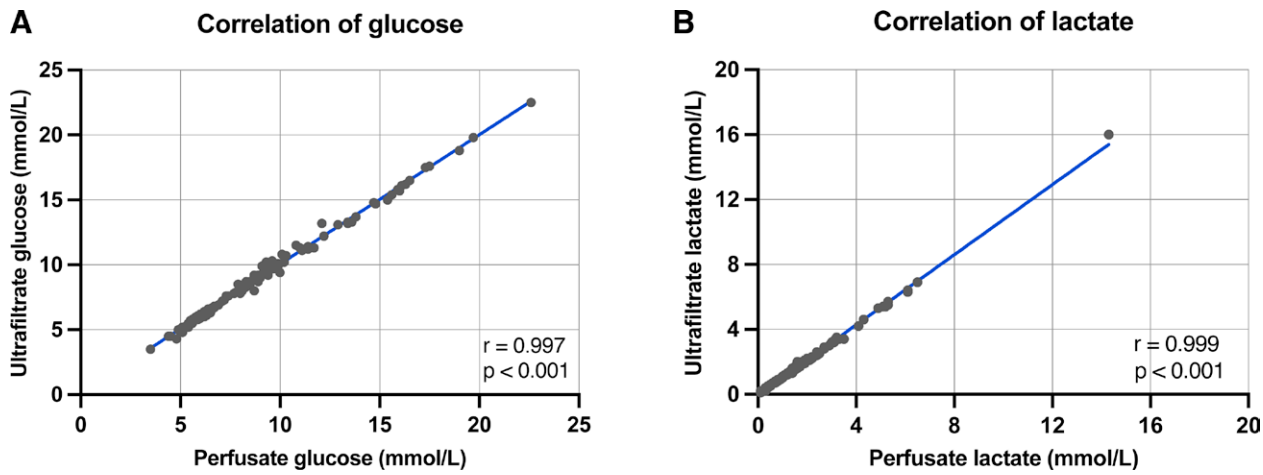

FIGURE 5. Correlation plot of (A) glucose values and (B) lactate values between the perfusate and the ultrafiltrate.

ultimate effects on outcome after transplantation remain to be demonstrated. We demonstrated a correction of the perfusate to more physiological values; however, the composition of the most optimal perfusion solution is yet unknown.

For long-term (multiple days) NMP, the use of CRRT is customary,[9,15] but for short-term NMP until now it is not. In some clinical NMP programs, short-term NMP can take up to 24 h, whereas, after 7 h, the perfusate becomes less physiological and might potentially be harmful to the liver. One may, therefore, question whether CRRT should also be incorporated into the NMP circuit for clinical, short-term NMP, at least for perfusions of >6 h. For long-term NMP, currently performed in a research setting, CRRT should be incorporated to minimize fluctuations and maintain a near-human physiological environment. We expect that this technique will probably be essential for prolonged perfusions for future repair and regeneration purposes.[26]

CONCLUSION

Already after 7 h of NMP, the perfusate reached increasingly high urea levels, resulting in a supraphysiological osmolarity. Although not routinely incorporated into currently available commercial NMP devices, the combination of CRRT with NMP substantially improved the perfusate composition to near-physiological values and might potentially improve posttransplantation outcomes in the recipients. For long-term NMP, CRRT is essential to maintain a stable and physiological milieu intérieur. Because most types of CRRT are relatively expensive and complex to integrate with the NMP circuit, we used a relatively cheap and simple setup with a small dialysis filter and roller pumps that ensure similar filtration and substitution flow rates. This setup was successfully used for long-term NMP for up to a week.

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