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Perfusion Pressures and Weight Loss During Normothermic Machine Perfusion of Human Donor Livers

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ABSTRACT

Background: Normothermic machine perfusion (NMP) is increasingly used to preserve and assess donor livers prior to transplantation. Due to its success, it is expected that more centers will start using this technology. However, NMP may also cause adverse effects.

Methods: In this retrospective, observational study, we investigated the effect of NMP pressures on donor liver weight, post-transplant outcomes, and hepatic perfusion characteristics. A total of 36 livers were transplanted after NMP. NMP perfusion pressure settings were lowered from a median (IQR) of 47 mmHg (42–54) to 34 mmHg (30–39) for the hepatic artery (HA), and from 8 mmHg (7–10) to 7 mmHg (6–8) for the portal vein (PV) to diminish potential edema formation inside the liver.

Results: This change appeared to lead to a reduction of liver weight after NMP (−22 g to −143 g, $p = 0.02$), without affecting the PV flow velocity (35.5 to 48.0 cm/s, $p = 0.54$), or hepatocellular injury markers during NMP (AST 1511–1148 U/L, $p = 0.44$; ALT 318–849 U/L, $p = 0.35$), and post-transplantation outcomes. Changes in liver weight correlated significantly with the applied PV pressure during NMP ($r = 0.52$, $p < 0.01$) and the HA flow ($r = 0.38$, $p < 0.05$).

Conclusion: NMP can lead to a reduction in liver weight, which might be masked by edema when high perfusion pressures are used. We encourage applying the lowest perfusion pressures possible to reach adequate flows and oxygen supply during liver NMP.

1 | Introduction

The current gold standard treatment for end-stage liver disease is transplantation. However, the continuing shortage of suitable donor organs has led to increased use of marginal (high-risk) livers from extended criteria donors (ECDs) [1, 2]. To enlarge the pool of donor livers, various techniques are being implemented, including ex-situ normothermic machine perfusion (NMP) of previously declined (and otherwise discarded) human livers [3]. NMP enables preservation and

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; COR, controlled oxygenated rewarming; CT, computer tomography; DUS, Doppler ultrasound; ECD, extended criteria donor; HA, hepatic artery; IPE, intrahepatic perivascular edema; NMP, normothermic machine perfusion; PAS, periodic acid-Schiff; PV, portal vein; RI, resistive index; SCS, static cold storage; SvO_2, venous oxygen saturation.

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assessment of ECD liver viability, potentially increasing the number of suitable livers available for transplantation [4, 5]. The transplantation of NMP-tested livers has been a considerable success [4–6], and it is expected that more centers will start using this technology in the near future.

However, it is also equally important to gain insight into the possible adverse effects of NMP on liver grafts. Richards et al., for instance, described the formation of a "cradle sign," a compression injury to the liver parenchyma due to its positioning against the plastic basket during short-term NMP (<24 h) [7]. Fortunately, this "cradle sign" appeared to have no effect on liver function and the success of liver transplantation. Another possible adverse effect is the formation of intrahepatic perivascular edema (IPE) in NMP liver grafts due to high perfusion pressures. After starting the NMP program in our hospital, we noticed an unexplained increase in the need for CT scans during the first week after transplantation for recipients with a liver graft perfused with NMP (3/12; 25%), compared to liver grafts stored on static cold storage (SCS) in the period 2019–2022 (6/48; 13%; χ^2 [1, $N=60$] = 1.18, $p=0.28$). These were the number of CT scans needed to verify the intrahepatic arterial flow after the arterial flow was either abnormal or nondetectable on the protocolized Doppler ultrasound (DUS) on postoperative day 1, 4, or 7, while the CT scans demonstrated a patent, bilateral, hepatic arterial flow. Additionally, these CT scans typically showed IPE around the larger vessels within Glisson's capsule, measured along the right and/or left proximal portal vein (PV) branch(es). Although IPE had no apparent negative consequences for the recipient, it would be better to minimize IPE to avoid potential negative effects on the liver tissue and function, resulting from increased diffusion distance for oxygen, nutrients, and waste products, and possible cell damage [8–10].

While the increased number of CT scans post-transplantation was not statistically significant and could therefore be coincidental, based on the subjectively observed increased incidence of IPE in liver grafts perfused with NMP, we hypothesized that this might have been related to the hydrostatic pressure in the hepatic artery (HA) and/or PV during NMP. We, therefore, decided to lower the perfusion pressures during NMP. The aim of this study was to investigate the effect of lower perfusion pressures during NMP on perfusion-related parameters and post-transplantation outcomes.

2 | Materials and Methods

2.1 | Data Source and Study Population

We retrospectively studied the outcome of all NMP liver grafts transplanted between March 2019 and March 2022 in our center. After the livers were nationwide declined for transplantation by all transplant centers, the livers were offered to our center for viability assessment and possible transplantation. The data from the perfusion process and the liver grafts were collected from the prospectively maintained database. Recipient data was derived from a post hoc analysis of an observational cohort study (www.trialregister.nl—Trial NL6334), which was approved by the Medical Ethics Committee (METc 2014/77).

2.2 | Machine Perfusion

The process of liver procurement, the NMP procedure, and the decision to transplant liver grafts have been described previously [5, 11]. In brief, liver grafts were procured using a standardized procedure by a regional multi-organ procurement team in the Netherlands. After procurement, the liver was placed on SCS and transported to our center. Upon arrival, the liver was weighted and cannulas were inserted in the PV and HA at the back table before the start of machine perfusion. Excessive tissue removed during the back table on the machine was weighted and deducted from the original weight. For the machine perfusion procedures, a pressure-regulated perfusion device, the Liver Assist device (XVIVO, Groningen, the Netherlands), was used. The NMP was preceded by 1-h DHOPE and 1-h controlled oxygenated rewarming (COR), as described previously [5]. Between the DHOPE and COR, the liver was briefly removed from the machine to change the acellular University of Wisconsin machine perfusion solution (Carnamedica, Warsaw, Poland) to a perfusate containing an oxygen (O_2) carrier, in our case red blood cells [12]. The composition of the perfusate was carefully designed to have a physiological colloid osmotic pressure between 25 and 28 [13]. The perfusate composition was not changed during the time period of this study. DHOPE was performed at around 10°C with the PV pressure ≤5 mmHg and the HA pressure ≤25 mmHg, and oxygenated with 1 L/min 100% O_2 as described previously [5]. During COR, the temperature was gradually increased from 20°C to 37°C, and the PV and HA pressures were raised to a maximum of 11 mmHg and 70 mmHg, respectively. Throughout COR and NMP, an air/O_2 mixture was used to reach a perfusate pO_2 of 10–14 kPa and a venous oxygen saturation (SvO_2) of 55%–75%. After 150 min of NMP, it was decided whether or not to transplant the liver based on our established viability criteria [5]. When the liver was accepted for transplantation, the liver remained on the machine till the recipient was ready to receive the liver. The liver was disconnected from the perfusion machine and subsequently flushed with 2 L of University of Wisconsin cold storage preservation solution (Bridge to Life, London, United Kingdom). The liver was weighted and placed in a sterile bowl with the cold storage solution and ice for transfer to the recipient.

2.3 | Analyses

2.3.1 | Perfusate Analyses

During NMP, arterial, venous, and bile samples were collected every 30 min for gas analyses (ABL 90 Flex blood gas meter, Radiometer, Brønhøj, Denmark). Lactate and SvO_2 were used as marker of sufficient oxygen supply to the liver during NMP. Oxygen supply was considered sufficient when lactate was decreasing after the start of NMP and remained ≤1.7 mmol/L during perfusion, and SvO_2 remained >55%. The bile was collected under mineral oil (Sigma Aldrich, Darmstadt, Germany) in an Eppendorf tube (2 mL tubes, Sarstedt, Nümbricht, Germany) and immediately analyzed for pH, bicarbonate, and glucose as markers for the cholangiocyte viability (bile pH >7.45, difference [delta] between bile and perfusate pH, bicarbonate, and glucose) [5].

NMP perfusate samples were collected for clinical biochemistry (Clinical Chemistry Laboratory, UMCG, the Netherlands) at the start of NMP, after 150, 240, and 360 min, and at the end of NMP. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as hepatocyte injury markers to evaluate the effect of perfusion pressure adjustments during NMP. The osmolarity was calculated to observe its effect on the IPE formation, because of the possible effect on fluid shifts into the cells, using the following formula: 2× [sodium] + [glucose] + [urea].

2.3.2 | Radiology

Post-transplant, DUS examinations were performed routinely according to our institutional protocol on postoperative days 1, 4, and 7 by a dedicated team of specialized and experienced abdominal radiologists. There were no relevant changes in this team during the study period. In most examinations, a Fujifilm ARIETTA 850 ultrasound machine (Fujifilm, Tokyo, Japan) was used, with a 6 MHz convex transducer. The portal flow velocity was measured in the PV main branch, and the peak-systolic and end-diastolic arterial flow velocities were measured in the liver hilum. The resistive index (RI) was calculated as follows: (peak-systolic velocity—end-diastolic velocity)/(peak systolic velocity). In general, the postsurgical inflammation and edema are most pronounced on postoperative day 4 [14, 15]. For this reason, we focused on the postoperative day 4 DUS in this study.

2.3.3 | Histology

Biopsies were taken from the liver parenchyma during the preparation of the liver on the back table prior to DHOPE and after NMP, just before disconnecting the liver from the pump. Biopsies were fixated in 10% buffered formalin, processed, and embedded in paraffin. The paraffin-embedded biopsies were cut into 3–4 μm sections for later processing. After paraffin removal, slides were stained with periodic acid-Schiff (PAS) according to a standardized technique. PAS stains carbohydrates and can detect changes in glycogen storage in biopsies before and after NMP. Stained slides were digitally scanned (Hamamatsu, Japan) and processed using QuPath (v.0.3.0).

2.4 | Statistics

Continuous data were presented as the median and interquartile range (IQR). Because of fluctuations in the HA and PV flows during NMP, the mean flows, pressures, and SvO_2 during the perfusion were used for the median calculations. For the other parameters, the value at the moment of viability assessment (lactate and cholangiocyte viability values) or at the end of perfusion (AST, ALT, osmolarity) was used. The unpaired *t*-test (DUS data), the Mann–Whitney *U* test, or the one-way ANOVA were used for the comparison of continuous data between groups. Categorical data were expressed as numbers and percentages,

TABLE 1 | Machine perfusion characteristics for the three cohorts.

	P1 (*n* = 12)	P2 (*n* = 12)	P3 (*n* = 12)	*p* value all
Mean PV pressure (mmHg)	7.9 (7.1–9.5)	8.0 (7.1–9.0)	6.9 (6.1–7.9)	0.05
Mean HA pressure (mmHg)	47 (42–54)	43 (35–50)	34 (30–39)	**< 0.01**
Mean PV flow (mL/min/100 g)	108 (93–122)	96 (90–105)	86 (72–91)	**< 0.01**
Mean HA flow (mL/min/100 g)	34 (28–38)	30 (25–33)	23 (19–30)	**0.01**
Mean SvO_2 (%)	82.8 (79.3–88.3)	78.7 (71.1–85.4)	78.1 (75.4–83.4)	0.12
Lactate (mmol/L)[a]	0.4 (0.4–1.2)	0.4 (0.1–0.7)	0.5 (0.1–0.8)	0.80
Bile pH (mmol/L)[a]	7.54 (7.48–7.61)	7.50 (7.46–7.54)	7.51 (7.47–7.60)	0.32
Δ pH[a]	0.15 (0.09–0.24)	0.11 (0.06–0.16)	0.12 (0.08–0.25)	0.31
Δ Glucose (mmol/L)[a]	−11.1 (−14.0 to −5.7)	−5.5 (−8.4 to −4.6)	−8.9 (−11.7 to −6.3)	0.14
Δ Bicarbonate (mmol/L)[a]	9.0 (5.0–16.0)	9.0 (4.3–10.6)	8.4 (6.5–20.6)	0.15
AST (U/L)[b]	1511 (667–5224)	1089 (786–3759)	1148 (426–1855)	0.44
ALT (U/L)[b]	1366 (499–3417)	979 (596–2476)	849 (358–1332)	0.35
Perfusate osmolarity (Osmol/L)[b]	318 (307–321)	324 (310–343)	324 (310–330)	0.23
Change in liver weight (g)	−22 (−90 to 55)	−46 (−134 to 7)	−143 (−287 to −110)	**0.02**
Change in liver weight (%)	−1.1 (−5.2 to 3.3)	−2.4 (−8.5 to 0.5)	−10.2 (−16.5 to −6.1)	**0.02**
Duration NMP (min)	467 (437–513)	567 (478–643)	544 (486–591)	**0.01**
Graft utilization rate (No.)	12/23 (52%)	12/16 (75%)	12/16 (75%)	0.60

Note: Data are expressed as median and interquartile range or numbers and percentage. Δ indicating the difference between bile and perfusate values. The *p* values in bold are significant values.
Abbreviations: ALT, alanine amino-transaminase; AST, aspartate amino-transaminase; HA, hepatic artery; NMP, normothermic machine perfusion; PV, portal vein; SvO_2, venous oxygen saturation.
[a]At time point of viability assessment after 2.5 h of NMP.
[b]At end of NMP procedure.

and groups were compared using the chi-squared or Fischer's exact test. To determine the relationship between two variables, the Spearman nonparametric correlation test was used. A $p < 0.05$ was considered statistically significant. Analyses were performed using GraphPad Prism v10.0.02 (GraphPad Software, San Diego, CA, USA).

3 | Results

In total, 150 livers were transplanted in our center between March 2019 and March 2022, of which 36 were transplanted after NMP. The donor characteristics are presented in Table S1. The NMP livers were divided chronologically into three groups. The first period (cohort P1), the group that was found to have an increased number of CT scans made after transplantation (see Section 1), comprised livers perfused from March 30, 2019, to July 6, 2020 ($n = 12$); the second period (cohort P2) ranged from July 23, 2020, to April 29, 2021 ($n = 12$); and the third period (cohort P3) from June 1, 2021, to February 26, 2022 ($n = 12$). The only difference between the three cohorts in donor characteristics was the shorter cold ischemia time in the third time period, due to the start of the backable on the pump [16].

3.1 | Change in Applied Perfusion Pressures

During the study period, the median mean HA pressure applied during NMP was significantly reduced from 47 mmHg (42–54 mmHg) in P1 to 34 mmHg (30–39 mmHg) in cohort P3 ($p < 0.01$) (Table 1, Figure 1A). The median mean PV pressure was adjusted from 8 mmHg (7–10 mmHg) in P1 to 7 mmHg (6–8 mmHg) in cohort P3 ($p = 0.07$) (Table 1, Figure 1B). The need for a CT scan in the recipient because of difficulty visualizing HA on routine DUS after liver transplantation, declined over time. With CT scans needed in 3/12 (25%) recipients in P1, 2/12 (17%) CT scans in P2, compared to 1/12 (8%) CT scan needed in cohort P3 (Table 2). Also, some other post-transplantation outcomes improved between the three cohorts, such as a reduction in ICU and hospital stay (Table 2). However, we also observed a tendency to use the NMP livers in patients with a lower MELD score over time (Table 2). Additionally, in cohort 2, graft survival was lower compared to the other cohorts, although this was not statistically significant. Grafts were lost due to HA thrombosis, recurrent primary sclerosing cholangitis, and nonanastomotic biliary strictures. Unfortunately, one recipient died of drug-induced interstitial lung disease (everolimus).

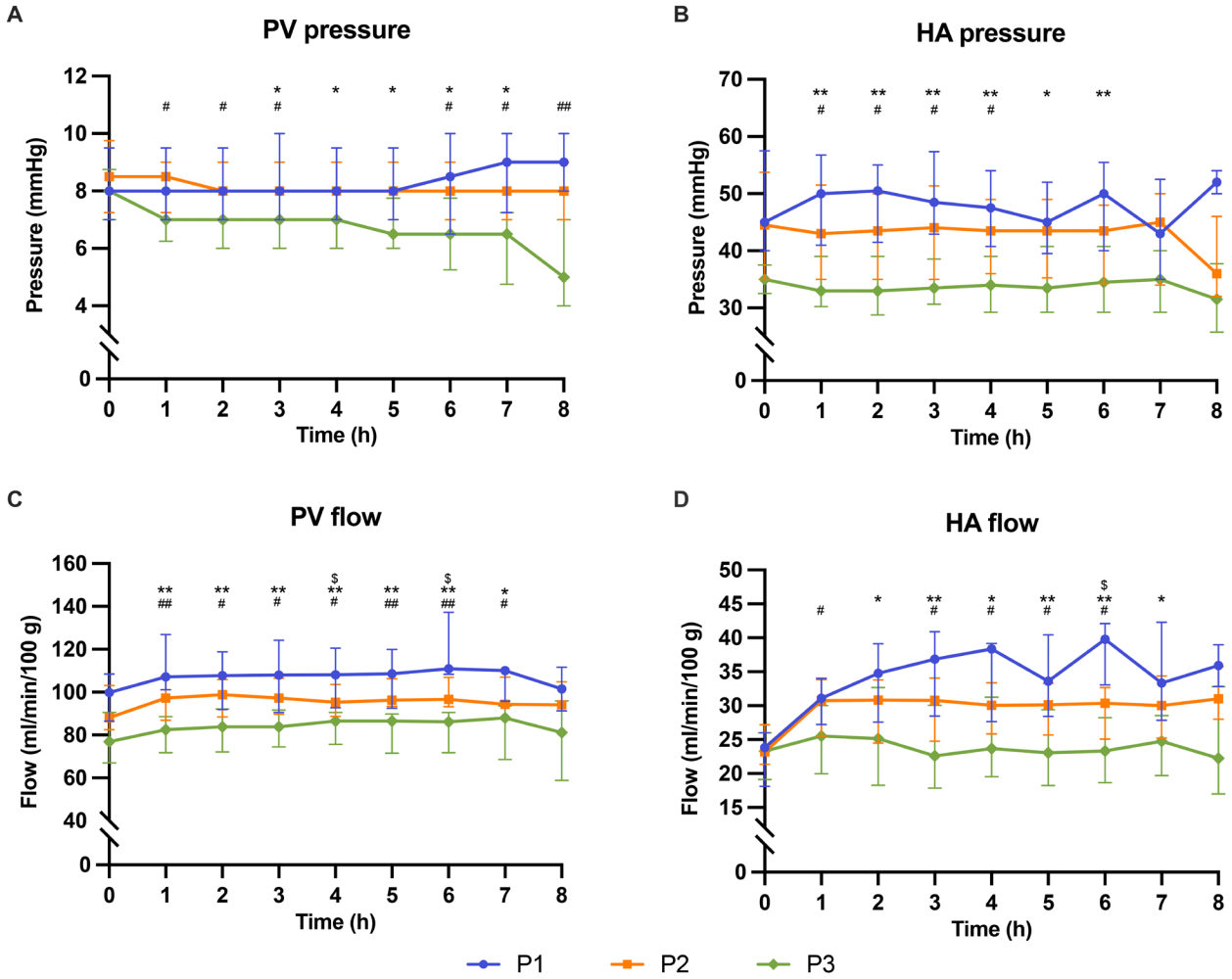

FIGURE 1 | Applied perfusion pressures and obtained flows during NMP in the 3 cohorts of NMP liver grafts. Presented are the medians with interquartile ranges. $Indicates a statistically significant difference between cohort P1 and cohort P2, *between P1 and P3, and #between P2 and P3, with the numbers of signs indicating the accuracy of the significance, for example: *$p < 0.05$, **$p < 0.01$. HA, hepatic artery; NMP, normothermic machine perfusion; PV, portal vein; T, time period. [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Effect on the Portal and Arterial Flow Velocity on Doppler Ultrasound

On postoperative day 4, the median velocity of the PV in the preanastomotic main branch was significantly higher in cohort P2 (55.0 cm/s [44.0–76.0 cm/s]) compared to cohort P1 (35.5 cm/s [29.8–51.2 cm/s]) ($p = 0.03$), and the same trend was seen between P1 and P3 (48.0 cm/s [42.0–75.6 cm/s]) ($p = 0.07$) (Figure 2). No differences were found in the flow velocities of the HA flows between the three cohorts (Figure 2).

3.3 | Consequences of Perfusion Pressure Changes

The decrease in pressures used during machine perfusion resulted in a significant reduction in flows. The median mean HA flow decreased from 34 mL/min/100 g (28–38 mL/min/100 g) in cohort P1 to 23 mL/min/100 g (19–30 mL/min/100 g) in cohort P3 (median absolute difference 11 mL/min/100 g; $p = 0.02$) (Table 1, Figure 1C). The median mean PV flow decreased from 108 mL/min/100 g (93–122 mL/min/100 g) in cohort P1 to 86 mL/min/100 g (72–91 mL/min/100 g) in

TABLE 2 | Recipient characteristics and post-transplant outcomes.

	Cohort P1 ($n = 12$)	Cohort P2 ($n = 12$)	Cohort P3 ($n = 12$)	p value all
Recipient characteristics				
Age (years)	63 (59–66)	59 (52–68)	64 (48–69)	0.94
Gender				
Male	8 (67%)	7 (58%)	7 (58%)	> 0.99
Female	4 (33%)	5 (42%)	5 (42%)	
Body-mass index (kg/m^2)	30 (24–34)	25 (23–30)	30 (28–32)	0.10
MELD-score	16 (10–17)	13 (9–18)	9 (7–11)	**0.03**
Transplant indication				
MAFLD	7 (58%)	4 (33%)	2 (17%)	0.17
Post-alcoholic cirrhosis	2 (17%)	2 (17%)	3 (25%)	
Biliary diseases[a]	2 (17%)	4 (33%)	1 (8%)	
Other[b]	1 (8%)	2 (17%)	6 (50%)	
Post-transplant outcomes				
Graft survival at 1 year[c]	11 (92%)	8 (73%)	12 (100%)	0.10
Patient survival at 1 year[c]	12 (100%)	11 (92%)	12 (100%)	> 0.99
Biliary complications[d]	6 (50%)	7 (58%)	5 (42%)	0.91
Primary nonfunction	0 (0%)	0 (0%)	0 (0%)	> 0.99
Acute rejection	0 (0%)	0 (0%)	0 (0%)	> 0.99
Chronic rejection	1 (8%)	0 (0%)	0 (0%)	> 0.99
Peak AST (UI/L)	1045 (784–1375)	1095 (717–2714)	884 (425–1644)	0.27
Peak ALT (UI/L)	754 (554–942)	610 (525–980)	524 (325–956)	0.46
Postoperative ICU stay (days)	2 (2–5)	1 (1–2)	1 (1–1)	**0.01**
Postoperative hospital stay (days)	20 (14–27)	15 (14–22)	10 (9–13)	**0.01**
Number of CT scans (No.)	3 (25%)	2 (17%)	1 (8%)	0.85
PV flow velocity preanastomotic main branch (cm/s)	35.5 (29.8–51.2)	55.0 (44.0–76.0)	48.0 (42.0–75.6)	0.54
HA flow velocity hilum (RI)	0.73 (0.60–0.79)	0.69 (0.64–0.79)	0.69 (0.65–0.85)	0.34

Note: Continuous data are presented as median (IQR), categorical data as number (%). The statistical tests were not powered due to small sample size, these results should be interpreted with caution. The p values in bold are significant values.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CT, computer tomography; HA, hepatic artery; ICU, intensive care unit; MAFLD, metabolic-associated fatty liver disease; MELD, model for end-stage liver disease; PV, portal vein.

[a]Primary sclerosing cholangitis, and primary biliary cholangitis.

[b]Postviral cirrhosis, hepatocellular carcinoma, cryptogenic cirrhosis, polycystic liver disease, and colorectal carcinoma hepatic metastases.

[c]Cohort 1: chronic rejection; Cohort 2: hepatic artery thrombosis, recurrent primary sclerosing cholangitis, nonanastomotic biliary strictures, and one patient dead due to drug-induced interstitial lung disease (everolimus).

[d]Cohort 1: Bile leakage (2×), anastomotic strictures (4×); Cohort 2: Bile leakage (3×), anastomotic strictures (3×), and nonanastomotic biliary strictures (1×); Cohort 3: Bile leakage (1×), anastomotic strictures (4×).

P3 (median absolute difference 22 mL/min/100 g; $p < 0.01$) (Table 1, Figure 1D).

The median mean SvO_2 decreased from 82.8% (79.3%–88.3%) in P1 to 78.1% (75.4%–83.4%) in P3 ($p = 0.09$) (Table 1, Figure 3A). However, SvO_2 remained > 55%, indicating that oxygen delivery to the liver remained sufficient. In line with this, the application of lower perfusion pressures did not have an impact on lactate clearance (Table 1, Figure 3B), cholangiocyte viability criteria markers (Table 1, Figure 3C–F), or graft utilization rate (Table 1). Moreover, when comparing cohorts P1 and P3, there were no significant differences in perfusate levels of the hepatocellular injury markers AST (1511 U/L [667–5224 U/L] versus 1148 U/L [426–1855 U/L]; $p = 0.31$) and ALT (1366 U/L [499–3417 U/L] versus 849 U/L [358–1332 U/L]; $p = 0.18$) (Table 1, Figure 3G,H).

3.4 | Change in Liver Weight During NMP

Most livers lost weight during NMP. While the median change in liver weight before and after NMP was slightly negative in cohort P1 (−22 g [−90 to 55 g] or −1.1% [−5.2% to 3.3%]), the weight loss significantly increased from cohort P2 onwards, with a median weight change in P3 of −143 g (−287 to 110 g) or −10.2% (−16.5% to 6.1%) (Table 1, Figure 4A,B). Change in liver weight (%) was significantly correlated with the applied PV pressure ($r = 0.52$; $p < 0.01$), and HA flow ($r = 0.38$; $p < 0.05$) (Figure 4C,D), but not with the applied HA pressure ($r = 0.10$; $p = 0.61$), and the PV flow ($r = 0.37$; $p = 0.05$) (Figure 4E,F). In other words, with decreasing HA and PV perfusion pressures, we observed more pronounced liver weight loss during NMP.

To determine whether liver weight loss could be explained by increasing perfusion time or increasing osmolarity of the perfusion fluid during NMP, we next examined differences in perfusion duration and osmolarity. The duration of the perfusion has significantly increased between cohort P1 and P2 (median absolute difference 100 min; $p = 0.02$) and P3 (median absolute difference 23 min; $p = 0.01$), but did not show a significant correlation between change in liver weight and NMP duration

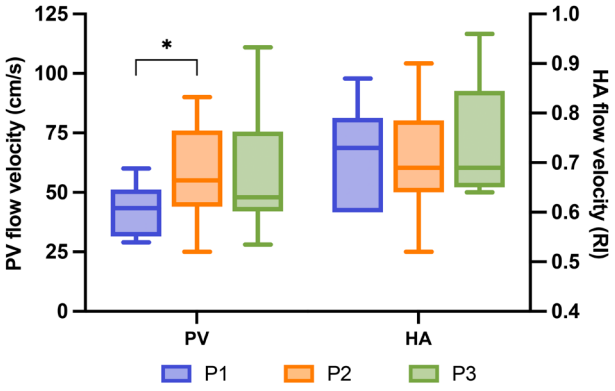

FIGURE 2 | Flow velocity in the portal vein and hepatic artery measured by postoperative Doppler ultrasound in the three cohorts. Portal flow velocity increased over time, while arterial flow velocity remained stable. *A statistically significant difference of $p < 0.05$ between cohort 1 and cohort 2. HA, hepatic artery; PV, portal vein. [Color figure can be viewed at wileyonlinelibrary.com]

(r = 0.17; $p = 0.40$) (Figure 5A,B). In general, perfusate osmolarity increased during NMP (Figure 5C). However, there were no significant differences in end-NMP osmolarity between the P1 and P3 (Table 1). Moreover, there was no significant correlation between end-NMP perfusate osmolarity and change in liver weight ($r = 0.11$; $p = 0.63$) (Figure 5D).

3.5 | Histology

Glycogen storage is accompanied by water retention [17]. For this reason, a loss in liver weight could be explained by a loss of glycogen storage. Interestingly, intrahepatic glycogen storage, as visualized by PAS staining, increased during NMP in 10 (77%) of the 13 livers with a biopsy. Only one liver (8%) exhibited a decrease in glycogen storage in the post-NMP biopsy, compared to the pre-NMP biopsy (Figure 6).

4 | Discussion

In recipients of an ex situ NMP-preserved liver, we had subjectively noted an increased need for CT scans because of inadequate visualization of the intrahepatic artery branches on routine DUS in the early post-transplant period. Although CT scans demonstrated patent arteries in all cases, pronounced edema was noted around the major vascular branches of livers after NMP, within Glisson's capsule. Edema can have several causes and is also seen after livers preserved with SCS [18]. However, this perivascular edema prompted us to apply a stepwise decrease in perfusion pressures during ex situ NMP to avoid potential negative effects on liver perfusion and/or function. Despite this reduction of perfusion pressures during NMP, we have observed adequate oxygenation of the livers, as illustrated by the absence of differences in hepatobiliary viability and injury markers or in utilization rate. This study indicates that the previously published perfusion pressure settings for ex situ NMP may be too high, and lower values can be applied while maintaining adequate flows and oxygen delivery.

Machine perfusion is increasingly used in clinical liver transplantation, and many centers are adopting this new technology as an improved preservation method for ECD livers. Although several studies have described the benefits of both hypothermic and NMP, very few studies have focused on potential side effects. The Cambridge group previously described the risk of pressure necrosis due to the positioning of a liver graft against the plastic basket of the liver container during NMP [7]. We here report another potential risk of NMP: perivascular edema of the major vascular branches in the liver, possibly due to fluid extravasation during or after NMP. When we initiated our machine perfusion protocol in 2012 [19], we intended to mimic the in vivo situation as close as possible. We, therefore, applied a mean arterial pressure of 50 mmHg and a portal venous pressure of 11 mmHg in our pressure-controlled perfusion device. While this has resulted in satisfactory results and a clinical protocol that has been adopted by many centers worldwide, we have learned over the years that lower perfusion pressure settings can and should be applied during ex situ NMP. We now advise a hepatic arterial pressure of around 35 mmHg and a portal pressure of 7 mmHg. In fact,

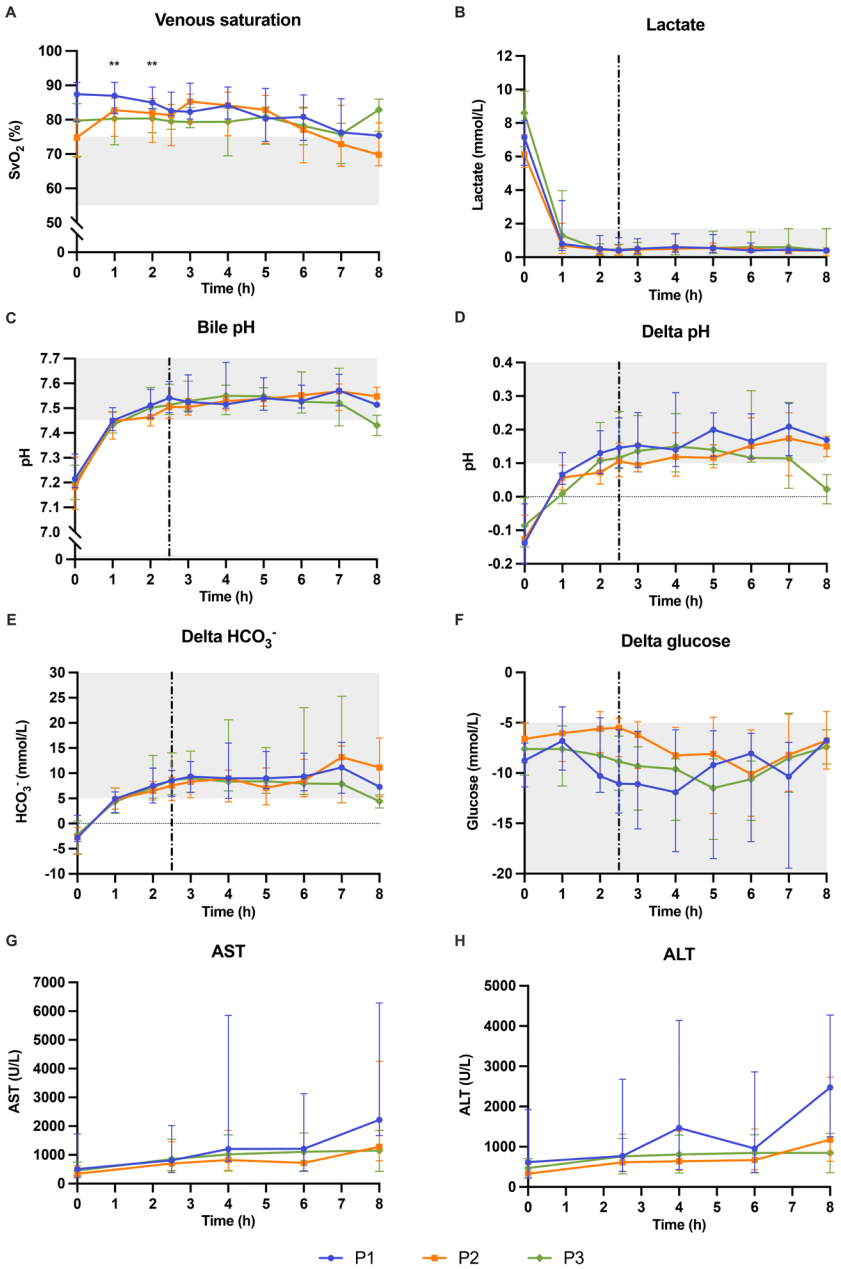

FIGURE 3 | The effect of adjustments in machine perfusion pressure on various markers of hepatobiliary function or injury. The gray-shaded areas indicate the desired SvO_2 value and the viability criteria values. The vertical interrupted line represents the time of viability assessment. (A) SvO_2 of the perfusion fluid. The difference in SvO_2 at the start of NMP between the cohorts can be explained by the higher fractional inspired O_2 used in cohort P1 (30%–100%), compared to the later cohorts P2-3, where the fractional inspired O_2 was set at 21%–30%. (B) Perfusate lactate clearance. The slightly higher lactate level at the start of NMP in cohort P3 can be explained by the fact that livers were flushed with normal saline prior to NMP in cohorts P1-2 and with Ringer's lactate solution in cohort P3. (C, D) Established biomarkers of bile duct viability with delta indicating the difference between bile and perfusate values. (E, F) Perfusate levels of aspartate AST and ALT during NMP. **A statistically significant difference of $p < 0.01$ between cohort 1 and cohort 3. ALT, alanine transaminase; AST, aspartate transaminase; NMP, normothermic machine perfusion; O_2, oxygen; SvO_2, venous oxygen saturation. [Color figure can be viewed at wileyonlinelibrary.com]

perfusion flow and oxygen delivery are more important than perfusion pressure.

An explanation of why lower perfusion pressures can be applied during ex situ NMP, compared to in situ values, could be the lower outside pressure on a liver when exposed to ambient air in a perfusion device instead of situated intra-abdominally in a living person. Moreover, perfusion flow depends on both pressure and the viscosity of the perfusion fluid. The perfusion fluid used during our NMP procedures typically has a relatively low viscosity with a hematocrit of around 0.19 L/L. With viscosity values that are lower than values of whole blood, a lower pressure can also be applied to obtain adequate flows and oxygen delivery. In the current study, the stepwise reduction in perfusion pressures during NMP resulted in a reduction of the perfusion flows (HA 24–27 mL/min/100 g; PV 88–91 mL/min/100 g) but did not have any notable harmful effect on the liver. There were no significant

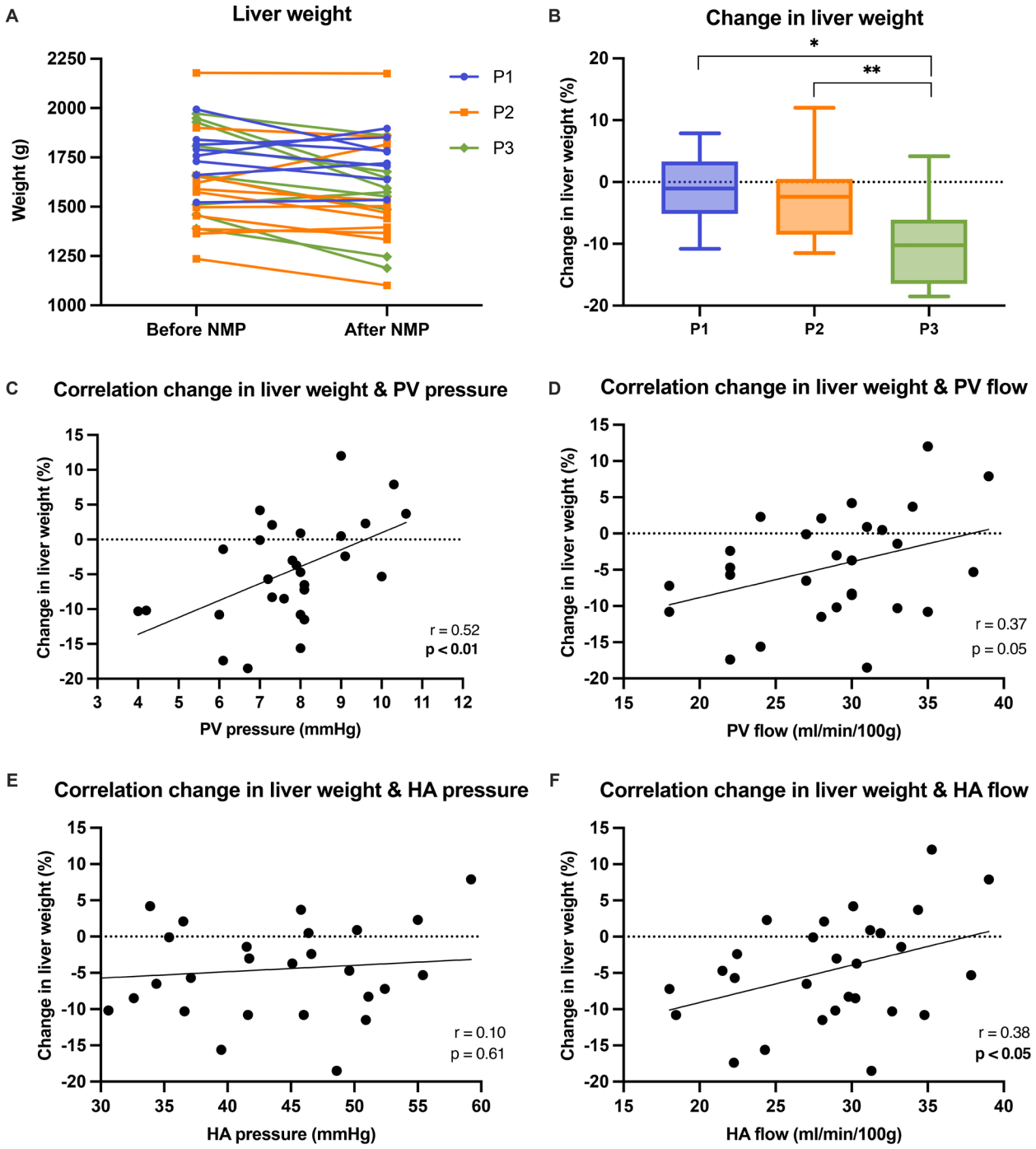

FIGURE 4 | Changes in liver weight and HA and PV pressures and flows during normothermic machine perfusion (NMP). (A, B) Changes in weight of each individual liver and in the three cohorts during NMP. Most livers lost weight during NMP. There was a significant change in weight loss between cohort P1 and cohort P3 ($p < 0.01$) and between cohort P2 and P3 ($p < 0.01$), but not between cohort P1 and P2 ($p = 0.51$). (C) Correlation between PV pressure and change in liver weight during NMP. (D) Correlation between PV flow and change in liver weight during NMP. (E) Correlation between HA pressure and change in liver weight during NMP. (F) Correlation between HA flow and change in liver weight during NMP. *A statistically significant difference of $p < 0.05$ between cohorts 1 and 3. **A statistically significant difference of $p < 0.01$ between cohorts 1 and 2. [Color figure can be viewed at wileyonlinelibrary.com]

differences in SvO_2, lactate clearance, cholangiocyte viability criteria markers, injury markers, and the utility rate among the three cohorts. The SvO_2 was slightly lower when applying reduced pressures; however, it remained within an acceptable range, and lower oxygen tension might even be beneficial, as hyperoxia during NMP can be a cause of postreperfusion syndrome and vasoplegia in the recipient [20]. Lactate clearance, cholangiocyte viability criteria markers, and injury markers did not differ between the three cohorts with adjusted pressures, indicating that the liver grafts did receive sufficient oxygen.

Despite the subjectively observed perivascular edema in livers after NMP, we also noted that, paradoxically, most livers lost

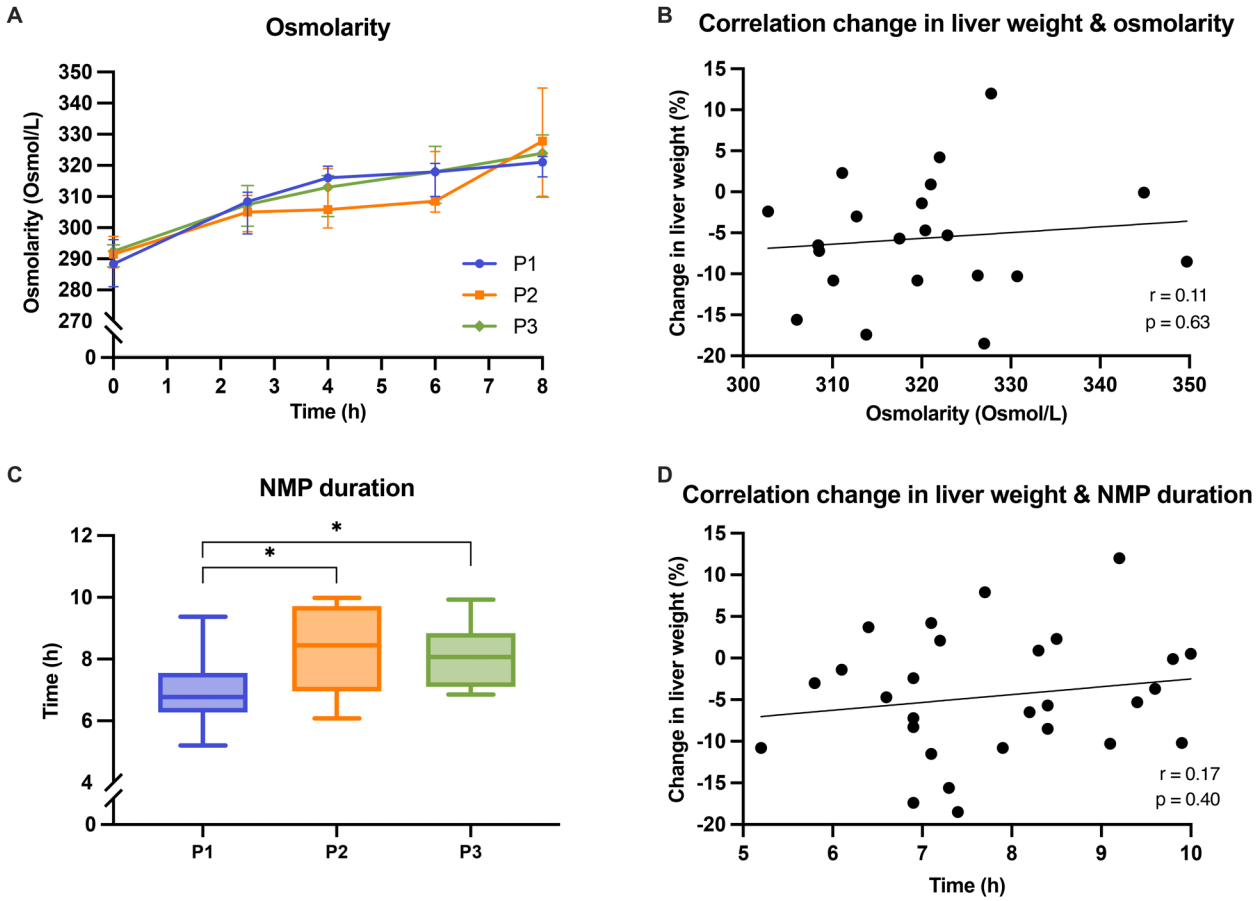

FIGURE 5 | Perfusate osmolarity during normothermic machine perfusion (NMP) and duration of NMP. (A, B) Perfusate osmolarity increased steadily during NMP, without significant differences between the three cohorts. Changes in liver weight did not correlate with perfusion fluid osmolarity at the end of NMP. (C, D) The duration of the perfusion was significantly different between cohort P1 and cohort P2 ($p = 0.02$) and P3 ($p = 0.01$), but not between P2 and P3 ($p = 0.48$). No correlation was found between changes in liver weight and the duration of NMP. *A statistically significant difference of $p < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

weight during NMP. Although we cannot rule out some weight loss during the DHOPE and COR phases (1 h each), the NMP phase has a substantially longer perfusion duration (8–10 h) at higher pressures and with higher flows. After applying lower perfusion pressures during NMP, this weight loss became even more pronounced. A potential explanation for this paradoxical effect could be that weight loss occurs in the parenchyma of the liver, while the edema is formed around the large vascular pedicles entering the liver. During the earlier NMP procedures (cohort P1), the reduction in liver weight was probably masked by the development of IPE, and only when IPE was reduced by applying lower perfusion pressures did the weight loss become more obvious (cohort P3).

The reduction in weight of the liver grafts during ex situ NMP has also been observed by other researchers [21], but so far, no good explanation for this has been found. We initially hypothesized that the weight loss could be caused by the increasing osmolarity of the perfusion fluid during NMP. Due to the accumulation of substances such as urea in the perfusion fluid during NMP, osmolarity may rise to supraphysiological values, potentially causing osmotic dehydration and shrinkage of cells [22]. However, we found no correlation between the change in liver weight and perfusion fluid osmolarity. Another cause of parenchymal weight reduction can be the depletion of intrahepatic glycogen storage. For every gram of loss of glycogen, 3 g of fluid can be lost [17], leading to a reduction in liver weight. However, we noted increased glycogen stores in most livers during NMP, which would result in an increase rather than a decrease in liver weight. An alternative explanation for the observed reduction in liver weight could be a reduced metabolic demand in a liver during ex situ machine perfusion compared to the in vivo situation. Clavien et al. proposed that livers during NMP are somewhat "put at rest," which may result in a reduction of hepatocyte volume [21]. The phenomenon of liver weight loss during NMP is clearly still incompletely understood and requires more research into the underlying mechanisms as well as the clinical consequences.

Due to the aim of this manuscript, to demonstrate our learning process with NMP, and therefore the study design, this study has several limitations. One limitation of this study is the retrospective study design and the relatively small groups. Also, the groups are in chronological order, which can contribute to time and expertise bias. A main difference between the three groups was the lower MELD score in the recipients and the shorter CIT in the last group. The shorter CIT may have contributed to a reduction in liver weight, potentially by decreasing IRI; however, the NMP duration was the longest in

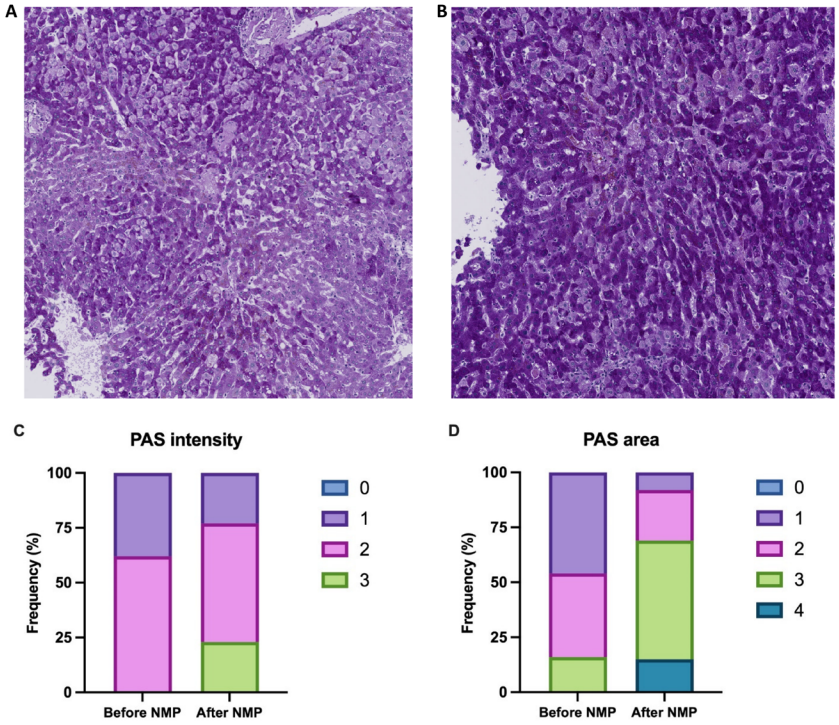

FIGURE 6 | Periodic acid-Schiff staining Examples of PAS staining (200 μm) of liver parenchyma biopsies taken before (A) and after NMP (B), indicating an increase in intrahepatic storage of glycogen during NMP. Both the intensity of PAS staining (C) and the area of PAS staining (D) increase during NMP. Scoring scale for PAS intensity: 0 = no PAS; 1 = light staining; 2 = moderate staining; 3 = maximal PAS staining. Scoring scale for area (1 cm^2) of PAS staining: 0 = no PAS staining; 1 = 1%–25% PAS staining; 2 = 26%–50% PAS staining; 3 = 51%–75% PAS staining; 4 = 76%–100% PAS staining. NMP, normothermic machine perfusion; PAS, Periodic acid-Schiff. [Color figure can be viewed at wileyonlinelibrary.com]

this group. The combination of a shorter CIT and longer NMP duration with low perfusion pressures might be the most beneficial. Additionally, a lower recipient MELD score could have contributed to the decrease in the number of CT scans performed in the recipient after transplantation. Nevertheless, we were able to demonstrate the safety of applying lower perfusion pressure settings during NMP than initially reported. Although our data support a role for perfusion pressure in the development of IPE, we did not scientifically measure this, and we cannot completely exclude other potential causes, such as a mismatch between oxygen demand and delivery. However, we did not find clinical evidence of such a mismatch. We used the Liver Assist device and have no comparable data with other devices. Therefore, we do not know whether the observed signs of IPE after NMP are also encountered when using other devices, and this would require further research. Another limitation is that we did not perform CT scans in all recipients of an NMP-preserved liver. Only patients with inadequate detection of arterial flow on routine DUS underwent CT scanning. Additionally, we did not quantify the amount of IPE on the scans. Furthermore, CT scans were not performed straight before or after reperfusion, which makes it more difficult to establish the cause of edema. Although the percentage of patients requiring a post-transplant CT scan decreased when applying lower perfusion pressures during NMP, we could not determine whether this indeed resulted in a reduction of the mean amount of perivascular edema for all patients, also because we did not quantify the amount of IPE on the scans. These limitations add extra uncertainty to our results; however, lower perfusion pressure appeared not to be harmful to the liver.

We did not find an explanation for the paradoxical liver weight loss during NMP, but the peripheral parenchymal biopsies used in this study only represent a small part of the liver grafts. Additional or more central biopsies might have given more information. The colloid osmotic pressure could also give more information on the increase in liver weight besides the osmolarity, but unfortunately, we were not able to measure the colloid osmotic pressure in the stored perfusate samples. Moreover, there is the possibility that livers increase in weight during SCS, which subsequently normalizes during machine perfusion. Therefore, to find an explanation for the observed weight loss during NMP, more phases of the transplantation process (procurement, transport, perfusion, and post-transplantation) should be investigated.

In conclusion, compared to our initial NMP protocol described a decade ago, we have stepwise decreased perfusion pressures during NMP and now apply pressure settings that are lower than normal in vivo values. Reduction in perfusion pressure during NMP was not associated with a reduction in oxygenation or diminished hepatic function or utilization rate. We, therefore, encourage to use the lowest perfusion pressures possible to obtain flows that provide sufficient oxygen supply while avoiding possible perivascular edema to avoid potential damage to the liver.

Author Contributions

Concept/design: Bianca Lascaris, Vincent E. de Meijer, and Robert J. Porte. Data collection: Bianca Lascaris, Silke B. Bodewes, and Adam M. Thorne. Data analysis/interpretation: Marius C. van den Heuvel,

Robbert J. de Haas, Bianca Lascaris, and Robert J. Porte. Drafting article: Bianca Lascaris. Critical revision of article and approval of article by all authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.