MolecularMedicine

Protective Mechanisms of Hypothermia in Liver Surgery and Transplantation

Pim B Olthof, Megan J Reiniers, Marcel C Dirkes, Thomas M van Gulik, Michal Heger, and Rowan F van Golen**

Department of Surgery, Surgical Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Hepatic ischemia/reperfusion (I/R) injury is a side effect of major liver surgery that often cannot be avoided. Prolonged periods of ischemia put a metabolic strain on hepatocytes and limit the tolerable ischemia and preservation times during liver resection and transplantation, respectively. In both surgical settings, temporarily lowering the metabolic demand of the organ by reducing organ temperature effectively counteracts the negative consequences of an ischemic insult. Despite its routine use, the application of liver cooling is predicated on an incomplete understanding of the underlying protective mechanisms, which has limited a uniform and widespread implementation of liver-cooling techniques. This review therefore addresses how hypothermia-induced hypometabolism modulates hepatocyte metabolism during ischemia and thereby reduces hepatic I/R injury. The mechanisms underlying hypothermia-mediated reduction in energy expenditure during ischemia and the attenuation of mitochondrial production of reactive oxygen species during early reperfusion are described. It is further addressed how hypothermia suppresses the sterile hepatic I/R immune response and preserves the metabolic functionality of hepatocytes. Lastly, a summary of the clinical status quo of the use of liver cooling for liver resection and transplantation is provided.

Online address: http://www.molmed.org
doi: 10.2119/molmed.2015.00158

INTRODUCTION

Liver resection is often the only treatment option with curative intent for patients with a primary or secondary hepatic malignancy. Major liver resection, however, is associated with a 90-d mortality rate of 5.8% (1) and a postoperative morbidity rate of 25.9% (2). Excessive blood loss in particular is associated with poor postoperative outcomes (3). To limit these risks, vascular inflow occlusion (VIO) is often applied during parenchymal transection. Whereas VIO effectively reduces blood loss, it concurrently cuts off the hepatic oxygen supply, which induces a variety of metabolic perturbations that predispose the liver to hepatic ischemia/reperfusion (I/R) injury once the inflow of oxygen is restored (see Molecular Aspects of Hepatic I/R Injury).

I/R injury predominantly results from the sustained metabolic demands of a warm ischemic organ and the lack of oxygen to meet these demands. A way to improve the liver's resilience to ischemia is to reduce organ temperature (4). As stipulated by the Arrhenius equation, the cellular metabolic rate is reduced by 50% for every 10°C drop in temperature. On the basis of this principle, hypothermia-induced hypometabolism has been used since the 1960s to protect liver grafts from extensive periods of ischemia. Whereas the use of liver cooling for liver preservation and transplantation purposes are well known (see Liver Transplantation), livers can also be cooled *in situ* during liver resection by perfusing the organ with a chilled solution through the afferent hepatic vasculature. This technique is known as *in situ* hypothermic perfusion (IHP) (5), and various adaptations of this technique have been recently used to improve ischemic tolerance during major liver surgery (see Liver Resection) (6,7).

Although the concept of liver cooling–induced hypometabolism is relatively straightforward, the (hepato)cellular response to hypothemic ischemia is not fully understood. This review therefore aims to elucidate how hepatocyte metabolism is affected by ischemia and to explain how hypothermia modulates these processes to reduce I/R injury. In addition, clinical advances in the use of hypothermia in liver resection and liver transplantation are summarized. These insights may expedite a wider implementation of hypothermia in major liver

---

**MH and RFvG contributed equally to this work.*

Address correspondence to *Pim B Olthof, Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Phone: +31-20-5668837; Fax: +31-20-6976621. E-mail: p.b.olthof@amc.nl.*
Submitted June 25, 2015; Accepted for publication October 23, 2015; Published Online (www.molmed.org) November 3, 2015.

surgery and may help to select cases that will most likely benefit from therapeutic liver cooling.

MOLECULAR ASPECTS OF HEPATIC I/R INJURY

Hepatic I/R injury comprises a sterile inflammatory response that follows hepatic ischemia and is characterized by overproduction of reactive oxygen species (ROS) followed by activation of the innate immune system (8,9). The pathophysiology of hepatic I/R is summarized in Figure 1. Considering the close relation between these processes, hepatic I/R injury can be divided into three distinct phases on the basis of the operant inflammatory mechanisms and the main source of ROS production (9).

During ischemia, the absence of oxygen leads to cessation of the oxidative phosphorylation-dependent formation of ATP, causing buildup of the electron transport chain (ETC) substrates reduced nicotinamide adenine dinucleotide (NADH) and succinate (10,11). Consequently, hepatocytes undergo a metabolic switch to anaerobic glycolysis to generate ATP, which yields insufficient ATP to maintain cellular energy stores and sustain liver homeostasis (11). The high levels of oxidative phosphorylation substrates favor ROS formation by hepatocyte mitochondria once the inflow of oxygen is restored (12). Occurring in the first ±15 min of reperfusion (11), this burst in ROS formation marks the hyperacute phase of I/R injury.

The surge in ROS formation activates both apoptotic (programmed) and necrotic (uncontrolled) cell death pathways through various mechanisms. First, ROS irreversibly oxidize ETC complexes (13), which impedes ATP production and further increases ROS production as a result of exacerbated ETC electron leakage. ATP depletion further leads to dysfunction of energy-consuming plasma membrane ion transporters such as the Na^+/K^+ ATPase. The consequent ion imbalance and the rise in intracellular $[Ca^{2+}]$ in particular can directly induce cell death due to osmotic swelling and plasma membrane disruption (that is, oncotic necrosis) or induce mitochondrial permeability transition (MPT) (14). MPT is characterized by an increase in permeability of the mitochondrial membrane, which results in leakage of mitochondrial constituents (for example, cytochrome c) into the cytosol and activation of apoptotic pathways (15,16). In hepatocytes that are stressed by ischemia, however, ATP depletion and ROS overproduction collectively decrease the threshold for MPT (17), which ultimately results in secondary necrosis, since these cells lack the energy (ATP) required to execute apoptosis. Although oncotic and secondary necrosis are morphologically and etiologically distinct, both types of cell death have similar consequences on I/R injury.

The main consequence of hepatocyte necrosis is the release of damage-associated molecular patterns (DAMPs) into the circulation, which are intracellular molecules that gain

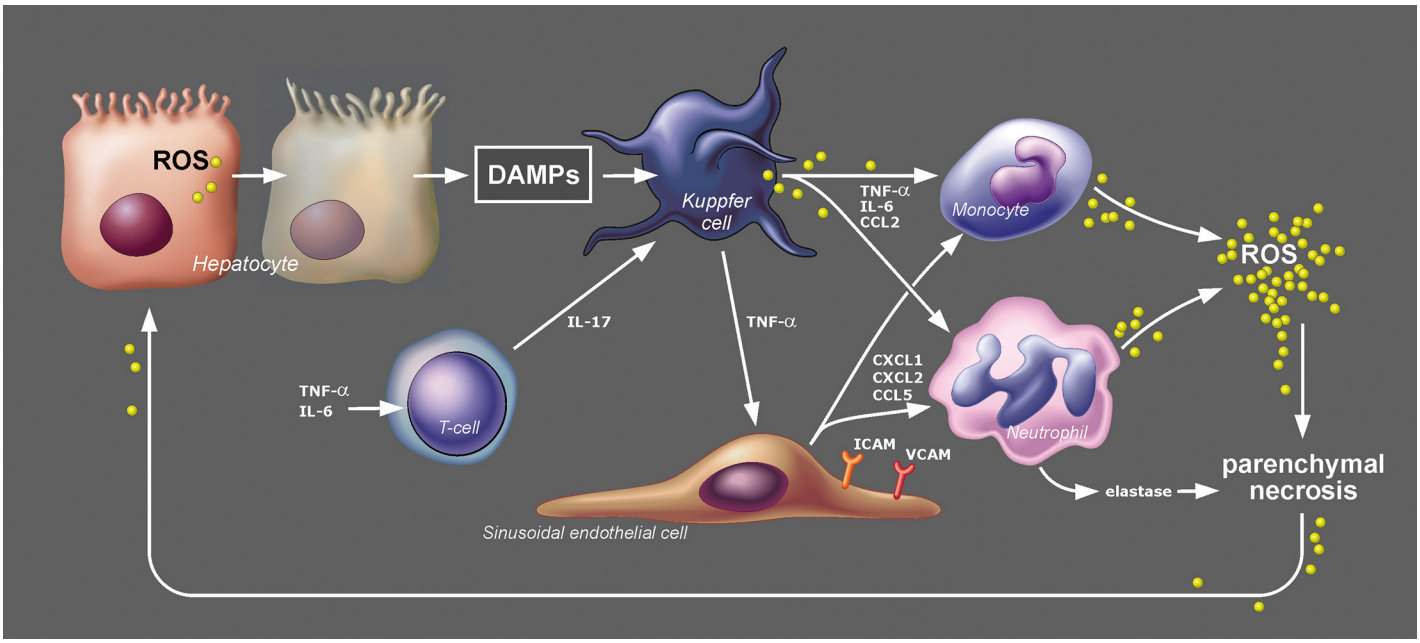

Figure 1. Molecular pathophysiology of liver I/R and the sterile immune response. Hepatocytes unable to cope with extensive ROS production undergo necrotic cell death and release DAMPs. These DAMPs activate Kupffer cells, leading to production of ROS, chemokines and cytokines that recruit additional leukocytes directly or via activated sinusoidal endothelial cells. The chemoattracted leukocytes (mainly monocytes and neutrophils) amplify ROS production, thereby inducing parenchymal necrosis and additional DAMP release. The shown processes should be interpreted in a cyclical manner and are reviewed in detail elsewhere (8,9).

immunogenic properties after their release and mobilize the innate immune system in the acute phase of reperfusion (approximately 15 min to 6 h of reperfusion). The sterile immune response that follows is characterized by chemoattracted leukocytes (mainly monocytes and neutrophils) that produce proinflammatory mediators, ROS, and protein-degrading enzymes in the chronic phase of reperfusion (>6 h) (Figure 1), which exacerbate I/R injury in concert with lymphocytes and platelets (18).

When the acute and chronic phases of reperfusion are severe, the loss of viable hepatocytes compromises liver function. Major parenchymal injury is reflected by an increase in circulating transaminases (AST and ALT) and reduced liver function, the latter of which is indicated by increases in prothrombin time (PT) and plasma bilirubin levels. These findings have been corroborated using quantitative liver function tests (19), which confirmed that severe I/R injury impairs liver function. Inasmuch as I/R injury also impairs liver regeneration, the ability to recover from surgery is further suppressed (19). If these effects persist, the ultimate consequence of I/R injury is postoperative liver failure, which is the most severe complication of liver surgery and is associated with high medical costs and a mortality rate of up to 88% (20).

PROTECTIVE MECHANISMS OF HYPOTHERMIA

Mitochondrial Metabolism

Ischemia and mitochondrial metabolites. The ETC consists of five enzyme complexes anchored in the inner mitochondrial membrane that facilitate energy production for cell metabolism. The electrons generated through oxidation of the tricarboxylic acid (TCA) cycle end products NADH (by complex I) and flavin adenine dinucleotide ($FADH_2$) (by complex II) are funneled into the ETC, producing energy that is used to establish an inward-directed proton gradient over the inner mitochondrial membrane. ATP synthase (complex V) transports protons back over the inner mitochondrial membrane and uses this proton-motive force to produce ATP. Because of the continuous shuttling of electrons, the ETC is a highly reducing environment predisposed to generate ROS.

During hepatic I/R, the ETC is the primary source of ROS in the hyperacute phase (see Molecular Aspects of Hepatic I/R Injury), which mainly results from the buildup of ETC substrates (for example, NADH) during ischemia (21). Ischemia leads to cessation of the ETC due to the lack of oxygen, which causes buildup of the complex I substrate NADH that in (warm) ischemic organs is continuously produced by the TCA. The high NADH concentrations directly mediate mitochondrial ROS production by promoting reduction of flavin mononucleotide at ETC complex I, which in reduced form reacts with O_2 to form ROS (12,22). More importantly, NADH buildup during ischemia also leads to ROS production during reperfusion through an indirect pathway that involves accumulation of the complex II substrate succinate (Figure 2).

Succinate is normally produced by the TCA from regular catabolites such as glucose and fatty acids. During ischemia, however, fumarate overload forces succinate dehydrogenase (ETC complex II) to work in reverse, thereby inducing succinate buildup. The overproduction of fumarate is caused by two different but converging metabolic pathways: the malate/aspartate shuttle (MAS) and the purine nucleotide cycle (11).

The MAS is a set of enzymatic reactions that transports reducing equivalents over the inner mitochondrial membrane, which is impermeable to NADH. To do so, cytosolic aspartate is first converted to oxaloacetate by aspartate aminotransferase. Oxaloacetate is next converted to malate by malate dehydrogenase in a reaction that concurrently converts NADH to NAD^+. Subsequently, malate is transported into the mitochondrial matrix and converted to oxaloacetate by malate dehydrogenase, thereby reducing NAD^+ to NADH. To complete the MAS, oxaloacetate is converted to aspartate by aspartate aminotransferase, which is then transported to the cytosol by the aspartate-glutamate antiporter (23).

During ischemia, the activity of the MAS is upregulated by high cytosolic NADH levels (Figure 2), which drives the formation of malate and fuels mitochondrial succinate accumulation. Because of the high cytosolic NADH/NAD^+ ratio, the conversion of oxaloacetate into malate is favored. Malate subsequently enters the mitochondrial matrix, where it would normally be converted to oxaloacetate to complete the MAS. This conversion, however, requires NAD^+, which is scarce during ischemia, since it is continuously used to produce ATP via anaerobic glycolysis (24). As a result, malate diverts from the MAS and is converted to fumarate instead of oxaloacetate by the NAD^+-independent enzyme fumarase. Fumarate is subsequently converted into succinate via reversal of succinate dehydrogenase activity. This reversal of succinate dehydrogenase is triggered because it results in oxidation of the ETC substrates $FADH_2$ and coenzyme Q, which both accumulate during ischemia (11).

The purine nucleotide cycle is the second source of substrate for succinate formation in ischemic tissue. During normothermic ischemia, the unavailability of oxygen means that ATP consumption exceeds ATP production, leading to the cytosolic breakdown of ATP to AMP, thereby producing energy. The purine nucleotide cycle preserves the end products of purine catabolism to enable rapid repletion of energy when oxygen becomes available again. The first step in this cascade is the deamination of AMP to inosine monophosphate, which is favored during ischemia. Inosine monophosphate is subsequently converted to adenylosuccinate by adenylosuccinate synthase, which in turn is transformed into AMP by adenylosuccinate lyase, producing fumarate as a byproduct. Fumarate is used to form succinate via the reactions described above for the MAS.

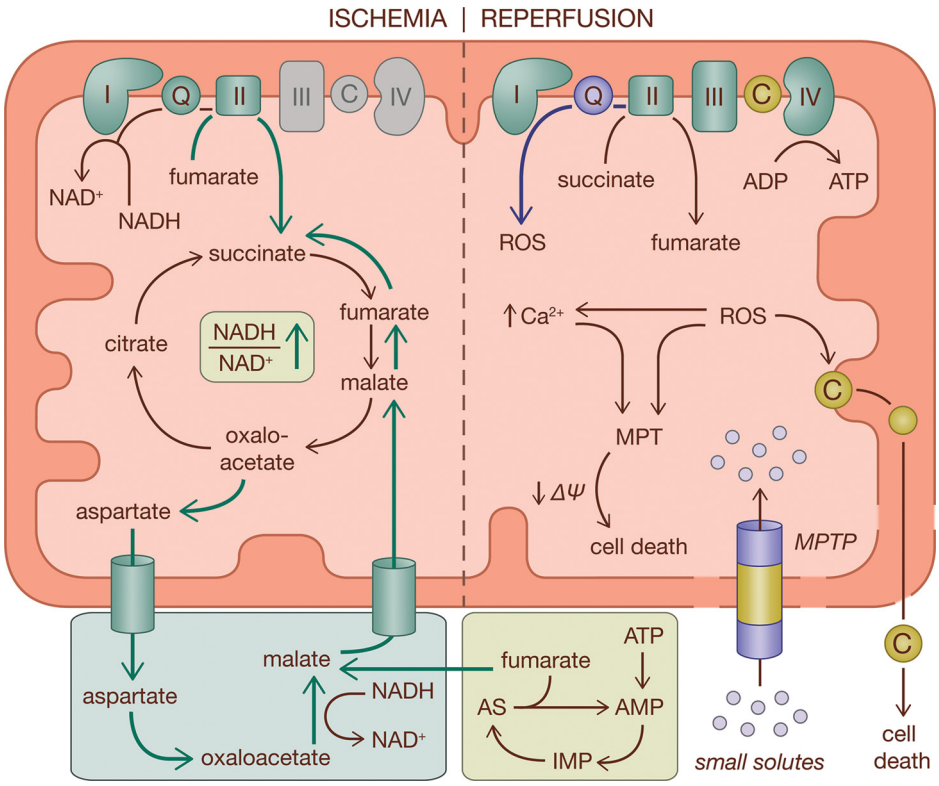

Figure 2. The molecular mechanisms of succinate-driven formation of ROS. During ischemia, the high NADH/NAD$^+$ ratio forces the MAS and purine nucleotide cycle (PNC) to produce excess fumarate (green arrows). Because of the high ubiquinol (QH_2) levels, succinate dehydrogenase (SDH, complex II) works in reverse and converts fumarate to succinate (green arrows). The accumulated succinate is rapidly oxidized during reperfusion, which leads to a surge in ROS production at complex I through reverse electron transfer (blue arrow). Excessive ROS production leads to MPT, collapse of the mitochondrial transmembrane potential ($\Delta\Psi$) and ultimately cell death. Additional details on the depicted signaling cascades are provided in the text (see Ischemia and Mitochondrial Metabolites). AS, adenylosuccinate; C, cytochrome c; IMP, inosine monophosphate; MPTP, mitochondrial permeability transition pore.

NADH and succinate that have accumulated during ischemia are rapidly oxidized by the ETC upon reperfusion to create a large proton gradient over the inner mitochondrial membrane and force ATP synthase to restore cellular ATP content. Because the magnitude of succinate oxidation during early reperfusion saturates the ETC, part of the electrons generated at complex II are forced back to complex I, where they form ROS in a process known as reverse electron transfer (11,12). Although most of the succinate-centered experiments were performed in I/R-subjected primary murine cardiomyocytes, the same metabolic responses to ischemia, including succinate buildup, were more pronounced in ischemic livers than in other ischemic organs (heart, kidney) (11). It is therefore likely that the mechanisms described above also apply to hepatic I/R injury.

Hypothermia and mitochondrial metabolites. Several studies have shown that suppressing mitochondrial metabolism during ischemia through liver cooling can reduce I/R injury. In a rat model of 45 min of partial hepatic ischemia performed under normothermic (37°C) or hypothermic conditions (34°C or 31°C), the hypothermia groups displayed less liver injury compared to the normothermic group. This was reflected by reduced hepatocellular necrosis, lower serum ALT/AST and attenuated neutrophil accumulation in the hypothermic groups. More importantly, the hypothermic groups had considerably lower circulating succinate levels compared to the normothermic group, as measured by nuclear magnetic resonance spectrometry (25). Similar results were found in obese Zucker rats subjected to 75 min of partial liver ischemia (26). Although the data suggest that hypothermia reduces succinate accumulation during ischemia, it is presently unclear how exactly systemic succinate levels relate to mitochondrial succinate buildup.

The harmful effects of purine catabolism during ischemia have also been effectively remediated with the use of therapeutic liver cooling. In a mouse model of 90-min left liver lobe ischemia, hypothermia (4°C to 32°C) reduced I/R injury, as evidenced by improved microcirculatory perfusion, a drop in plasma ALT/AST and less hepatocellular necrosis compared to the normothermic group. Considering that intrahepatic AMP concentrations and the total adenine nucleotide pool were preserved during hypothermic I/R, it was proposed that hypothermia conferred its protective effects by reducing AMP hydrolysis during ischemia (27), thereby limiting substrate availability for the ROS-generating hypoxanthine/xanthine system (28). Although not verified experimentally, the latter hypothesis is supported by the finding that liver I/R performed at 26°C lowered oxidative stress during reperfusion (measured as hepatic lipid peroxidation metabolites) (27). It was further shown that the protective effects of hypothermia were optimal when ischemic livers were cooled to 26°C, whereas lower temperatures did not impart additional hepatoprotection (29). This finding is in line with other reports (30) and suggests that a relatively modest decrease in liver temperature during ischemia is sufficient to reduce I/R injury. It is important to underscore,

however, that results obtained in mouse models using 60–90 min of ischemia might not adequately reflect the clinical situation. Sixty minutes of hepatic ischemia causes 75–100% hepatocellular necrosis in mice, which far exceeds the clinical hepatic I/R injury profile (31). Unfortunately, only few studies using milder injury (that is, shorter ischemia times) are currently available.

I/R and mitochondrial bioenergetics. It has been well established in several experimental models of liver I/R that ischemia depletes intracellular ATP levels (27). The depletion of cellular energy reserves during (normothermic) ischemia mainly results from the continuation of liver metabolism (ATP consumption) in the absence of ATP repletion via oxidative phosphorylation. ATP is therefore predominantly generated via anaerobic glycolysis during ischemia, which yields only a fraction of ATP compared with oxidative phosphorylation. The end product of glycolysis is lactate, which lowers cellular pH and reduces glycolytic enzyme activity, thereby inhibiting the main source of energy during ischemia (32). The high $NADH/NAD^+$ ratio that prevails during ischemia further reduces glycolytic activity because this process requires NAD^+ (32).

The mechanisms described under Ischemia and Mitochondrial Metabolites illustrate the pathways of ROS formation during hepatic I/R, which can compromise liver function during reperfusion by oxidizing proteins (33). Oxidative modification of amino acids in mitochondrial complex I, III and V has been observed in mouse livers subjected to I/R, leading to decreased ATP production during early reperfusion (34). A timely repletion of cellular ATP stores is crucial for livers to recover from an ischemic hit (10), which likely translates to amelioration of necrotic cell death when ATP stores are expediently restored. To fully complete apoptosis, 15–20% of physiological ATP stores are required (35). When ATP stores drop below these limits through the aforementioned mechanisms, the apoptotic process is redirected toward oncotic necrosis, thereby releasing DAMPs into the circulation (35). Reducing ROS formation during reperfusion by cooling livers during the ischemic phase may therefore reduce ETC complex oxidation and I/R injury by improving ATP synthesis and limiting the extent of sterile inflammation during reperfusion.

Hypothermia and mitochondrial bioenergetics. Several murine models of hepatic I/R injury have demonstrated that hypothermic liver ischemia preserves cellular ATP stores compared to normothermic ischemia (27,36). According to the Arrhenius equation, a 10°C reduction in temperature reduces metabolism by 50%. The preservation of ATP during hypothermia therefore results from a general reduction in hepatocyte metabolic rate and consequent reduction in ATP consumption rather than from an increase in ATP production (37). The lowered ATP demand under hypothermic conditions also counteracts the unfavorable metabolic switch to anaerobic glycolysis seen during normothermic ischemia. As a result, acidosis is avoided, which would otherwise be associated with metabolic dysregulation (38), and ATP depletion is deterred, which could otherwise lead to necrosis.

In a mouse model of 90 min of partial liver ischemia, hypothermia (4° to 32°C) effectively reduced lipid peroxidation, as evidenced by a drop in hepatic thiobarbituric acid reactive substances (TBARS) content (27). The reduction in ROS formation via the pathways described under Hypothermia and Mitochondrial Metabolites and the attenuation of lipid peroxidation suggest preserved ETC function and ATP production during reperfusion after hypothermic ischemia. In support of this premise, a more rapid recovery of hepatic ATP content was observed during reperfusion when rats cooled to 28°C were subjected to liver I/R versus normothermic controls (39).

During reperfusion, excessive ROS formation and ATP depletion leads to necrotic cell death, which impairs liver function and triggers inflammation through DAMP release. In a rat model combining 70% hepatic ischemia with resection of the nonischemic liver lobes, hypothermic (34°C) ischemia resulted in lower serum ALT/AST values compared with normothermia (37°C), which was accompanied by an increase in apoptotic cell death in the hypothermia group (40). These results support the hypothesis that preservation of cellular energy stores during hypothermia reduces the degree of necrosis in favor of apoptosis. A diminished extent of necrosis during severe hepatic I/R injury is associated with reduced DAMP release and liver inflammation (41).

Hypothermia and Immunomodulation

As mentioned under Molecular Aspects of Hepatic I/R Injury, hepatic I/R injury is characterized by a neutrophil-centered innate immune response. Several reports have indicated that hypothermia has strong hepatoprotective immunomodulatory effects. In a mouse model of 90 min of partial hepatic ischemia performed at either 37°C or ±25°C, neutrophil influx at 8 h of reperfusion was reduced by 99% in the hypothermic group. A similar extent of immunosuppression was observed in both hypothermic groups of a rat study comparing 45 min of partial hepatic ischemia at 37°C, 34°C and 31°C, thereby confirming that per-ischemic hypothermia can suppress the I/R immune response (26,42).

To explain this phenomenon, mRNA and serum levels of the neutrophil chemoattractants tumor necrosis factor (TNF)-α and chemokine (C-X-C motif) ligand 2 (CXCL2) were measured in the aforementioned mouse model, which were all reduced in mice subjected to I/R under hypothermic conditions (40,42). It was additionally shown that mice exposed to hypothermic I/R had reduced activation of the protein kinase c-jun N-terminal kinase (JNK) and the transcription factor activator protein 1 (AP-1), the latter of which is known to encode a host of I/R-pertinent cytokines (18,42).

The drop in cytokine production via suppression of JNK and AP-1 is most likely attributable to a hypothermia-mediated reduction in ROS formation after I/R, since both are activated by ROS (43). Corroboratively, adenoviral overexpression of the mitochondrial antioxidant mnSOD in mice diminished JNK activation and greatly reduced I/R injury (44). Although these results suggest that a hypothermia-induced reduction in ROS production accounted for the lowered JNK/AP-1 activation and consequent hepatoprotection, this hypothesis has not been validated experimentally.

In addition to the JNK/AP-1 axis, the reduction in innate immune signaling after hypothermic I/R could also be mediated by nuclear factor (NF)-κB, which is a transcription factor that, depending on the cell type, can promote either cell death and inflammation or cell survival and proliferation (33,42). After 90 min of hypothermic (<29°C) ischemia in mice, NF-κB was increased in hepatocytes, but decreased in Kupffer cells (KCs) (45). The decrease in KC NF-κB activation during hypothermia attenuated TNF-α production compared to the normothermic group, thereby reducing liver injury by avoiding post-ischemic neutrophil accumulation (45). The increase in hepatocellular NF-κB during ischemia is also advantageous, since it inhibits hepatocyte apoptosis and facilitates liver regeneration by suppressing JNK activity and by driving production of the mitogenic cytokines TNF-α and IL-6, respectively (46).

Heat shock proteins (HSPs) are the third molecular target of hypothermia with immunomodulatory properties. HSPs are chaperone proteins that can reduce inflammatory signaling by inhibiting cell death and preventing (leukocyte) NF-κB activation to protect organs from (ischemic) stress (47). Accordingly, HSP expression has been linked to milder liver injury in animal models of hepatic I/R injury (48). When 75 min of liver ischemia was combined with partial liver resection in rats operated at 37°C or 34°C, the hypothermic group displayed less hepatocellular injury (AST/ALT release) and survived longer than the normothermic group (40). The hypothermic group exhibited an upregulation of both HSP32 and HSP70, with a consequent decrease in TNF-α and CXCL2 production compared to normothermic I/R. These results suggest that HSPs help to silence the chemotactic signals that attract neutrophils after I/R (40).

Taken altogether, these data demonstrate that reducing the body temperature to <34°C has favorable effects on I/R-induced immune signaling. These protective effects include a reduction in hepatocyte JNK/AP-1 activity, a reduction in KC NF-κB expression and an increase in expression of cytoprotective HSPs, which in concert reduce neutrophil accumulation and consequent liver injury. The concurrent hypothermia-induced increase in hepatocyte NF-κB activation is also favorable, since NF-κB is known to govern hepatocyte proliferation and survival after I/R (49).

Hypothermia and Glucose Metabolism

Because the liver has a central role in glucose homeostasis, surgical trauma can lead to increased blood glucose levels (50). The relevance of the liver's role in glucose metabolism is underscored by the findings that postoperative hyperglycemia is associated with increased mortality after hepato-pancreatico-biliary surgery (51) and that early postoperative hyperglycemia has been identified as a risk factor for postoperative morbidity (52). In addition, hyperglycemia in liver surgery results in increased parameters of hepatocellular injury (53). In line with these observations in nondiabetic patients, diabetes is also an established risk factor for postoperative mortality in liver surgery (54). As such, strict control of blood glucose levels with insulin therapy in surgical ICU patients is crucial to improve clinical outcomes after major liver surgery (55).

Postoperative insulin resistance is the main cause of hyperglycemia after hepatic I/R (56) and might be related to inactivation of hepatic insulin receptors by JNK (57). As stated in Hypothermia and Immunomodulation, JNK is activated by TNF-α and ROS during hepatic I/R injury (58), after which JNK initiates a positive feedback loop that enhances its own activation by upholding TNF-α and ROS formation (33). Both TNF-α and ROS, however, can also induce hepatocyte insulin resistance independently of JNK (57). In line with these findings, other I/R-pertinent cytokines (for example, IL-6) have also been causally related to insulin resistance and postoperative hyperglycemia, albeit these effects occur through mostly unidentified mechanisms (18,57). Notwithstanding the elusive mechanisms, hepatic I/R injury does cause insulin resistance, glucose intolerance, hyperinsulinemia and a failure of insulin to suppress hepatic glucose production, ultimately resulting in a dangerous elevation of postoperative blood glucose levels (59).

In light of these findings, modulating JNK activation and cytokine signaling through hypothermia could improve hepatic insulin sensitivity and reduce hyperglycemia-related morbidity. Several reports have shown that hypothermia curtails the signaling cascades that derail glucose metabolism during and after surgery. First, the activation of JNK is reduced after 90-min partial hepatic ischemia in mice under hypothermic conditions, thereby neutralizing the most important trigger for insulin receptor inactivation (see Hypothermia and Immunomodulation) (42). Second, hypothermia has immunomodulatory effects, which could improve insulin sensitivity by lowering TNF-α and IL-6 levels (see Hypothermia and Immunomodulation). The effects of hypothermia on ischemia-induced perturbations in glucose metabolism were confirmed in a clinical study using liver cooling during major hepatectomy (Figure 3).

The fact that ketone body formation is also associated with poor clinical outcomes (60) further accentuates the importance of strict glycemic control in surgical patients. Ketone bodies are used

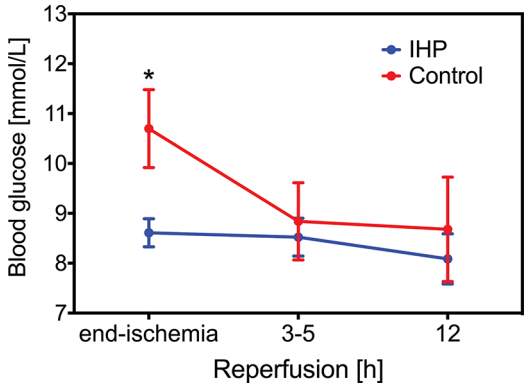

Figure 3. The effect of intraoperative liver cooling on blood glucose levels after major hepatectomy is performed under VIO. Patients scheduled for a right hemihepatectomy were randomized between intermittent VIO (control) or continuous VIO with liver cooling to a temperature of 28°C using IHP according to Reiniers *et al.* (7) (IHP, see Liver Resection). Blood glucose levels (*y* axis) were obtained by routine blood gas analysis and plotted as a function of reperfusion time (*x* axis). Values are displayed as mean ± standard error of the mean for nine IHP patients and five control patients. At the end of ischemia, blood glucose levels were higher in the control group than in patients treated with IHP (Mann-Whitney *U* test). The comparable glucose levels during the reperfusion phase are explained by the start of insulin therapy immediately after surgery. All study protocols (NCT01499979) were approved by the institutional review board.

by cells as an alternate energy source when glucose is unavailable and are produced when hepatic insulin signaling decreases, for example, in the case of insulin resistance. In a rat model of 45 min of partial hepatic ischemia induced at 37°C, elevated levels of the ketone body hydroxybutyrate were measured systemically at 24 h of reperfusion (25). This rise in ketone body formation could be attenuated by decreasing the body temperature of the rats to 31°C to 34°C during ischemia (25). Considering that hydroxybutyrate is formed from acetoacetate in a reaction that consumes NADH, the use of hypothermia probably normalized ketone body levels after I/R by reducing the NADH/NAD^+ ratio (see Hypothermia and Mitochondrial Metabolites) as well as by counteracting insulin resistance through the mechanisms described above.

The deleterious effects of metabolic perturbations after hepatic I/R are further underpinned by the increased susceptibility of obese, insulin-resistant Zucker rats to I/R injury (26,61). When obese or lean rats were subjected to 75 min of partial hepatic ischemia at either 37°C or 34°C (61), the hypothermic groups consistently exhibited less liver injury than the normothermic controls at 24 h of reperfusion, which was reflected by a reduction in transaminase release, improved liver histology and less endothelial injury. All obese rats subjected to hypothermic I/R survived the first 24 h of reperfusion, compared with only 20% in the normothermic control group (61). The marked protective effects of hypothermia on I/R injury in animal models of the metabolic syndrome imply that patients suffering from diabetes, (morbid) obesity or steatosis may especially benefit from therapeutic hypothermia during hepatic resections.

Hepatocyte Transporters

To fulfill the range of metabolic, synthetic and detoxifying tasks, hepatocytes are equipped with basolateral (for example, organic anion-transporting polypeptide [OATP], sodium-taurocholate cotransporting polypeptide [NTCP]) and canalicular (for example, MRP2) transport proteins that are respectively responsible for the uptake and biliary excretion of bile acids, drugs, and xenobiotics (62). Considering the severe hepatocellular injury induced by hepatic I/R, it is essential that the remaining viable hepatocytes provide sufficient liver function to avoid postoperative liver failure. The latter is supported by recent reports identifying bile acids as major regulators of liver regeneration after partial hepatectomy, which require a fully functional hepatocyte transporter machinery to convey their proliferative signals (63). Optimizing hepatocyte transporter function after surgery could therefore help patients to better recuperate from major liver surgery.

The effect of hepatic I/R injury on the basolateral OATP1B1, 1B3 and 2B1 transporters is best documented, mainly because these proteins are responsible for uptake of indocyanine green (ICG) and ^{99m}Tc-mebrofenin (64), which are used to quantitatively assess liver function (65). Functionality and expression of the rat homologs of these transporters (OATP1A1, 1A4, and 2B1, respectively [19]) was investigated in a rat model of partial liver ischemia combined with resection of the non-ischemic liver lobes. At 24 h after 30–60 min of partial liver ischemia, hepatocyte uptake function was diminished, as reflected by a marked drop in ICG and ^{99}Tm-mebrofenin clearance.

Although I/R injury could account for the reduced ^{99}Tm-mebrofenin clearance by triggering hepatocyte necrosis, it should be noted that I/R also suppressed *OATP1A1, 1A4* and *1B2* on a transcriptional level, which suggests a more refined control of transporter function during reperfusion (19). The first rationale for this finding was provided by the same study, which showed that the drop in *OATP* mRNA expression coincided with an increase in hepatic *TNFα* and *IL-6* transcript levels (19). These findings are substantiated by an earlier study in rats subjected to 60 min of liver ischemia, which revealed that the I/R-induced reduction in basolateral transporter (OATP1A1, 1A4 and 1B2) expression could be reversed by depleting KCs

before ischemia, thereby silencing cytokine production during reperfusion and supporting the premise that cytokines such as TNF-α directly downregulate OATP expression after liver I/R (66). The fact that several cytokines (TNF-α, IL-1β) are known to reduce the expression of other hepatocyte transporters (MRP2, NTCP) in various *in vivo* liver injury models further substantiates this claim. An alternate explanation for the downregulation of OATP expression during liver I/R might be the finding that exposure of cultured rat hepatocytes to hydrogen peroxide (that is, ROS) reduced the expression and/or transport function of OATP1A1, 1A4 and 2B1 (67).

Hypothermia might preserve transporter function after liver I/R. In a porcine model comparing hypothermic (4°C) with normothermic (38°C) liver ischemia (120 min), ICG clearance was unaltered after 24 h of reperfusion in the hypothermic group, whereas ICG clearance was markedly impaired in pigs that underwent normothermic liver ischemia (68). Although the intergroup differences in ICG clearance could merely reflect a hypothermia-mediated reduction in parenchymal cell death, the above-mentioned transcriptional downregulation of OATPs by cytokines and oxidative stress should also be kept in mind, especially since hypothermia effectively silences both pathological processes (see Hypothermia and Mitochondrial Metabolites, and Hypothermia and Immunomodulation). In that respect, it is also important to note that OATPs function independently of Na^+ and ATP (69), which means that the reduced ICG clearance and OATP dysfunction after I/R cannot be attributed to ischemia-evoked ion disturbances or ATP depletion. The latter further implicates cytokine signaling and/or oxidative stress in deterring OATP function after I/R, albeit the exact connection between these factors during liver I/R needs to be elucidated experimentally. It should be noted that hypothermia itself also affects transporter function and basolateral uptake of substrates, as we have shown for ICG in a series of pigs subjected to varying degrees of hypothermia (Figure 4). However, this effect is most likely of temporary nature, and transporter function is expected to recover to native conditions once the organ becomes euthermic during reperfusion, also benefitting from the protective effects of hypothermia.

In contrast to the basolateral OATPs, most canalicular transporters are members of the ATP-binding cassette transporter family (for example, multidrug resistance protein 2 [MDR2], multidrug resistance-associated protein 2 [MRP2]), which function at the expense of ATP (70). The reduction in ATP depletion and more rapid restitution of ATP stores during reperfusion as a result of hypothermia (see Hypothermia and Mitochondrial Bioenergetics) could therefore improve the function of these transporters during reperfusion. This is particularly

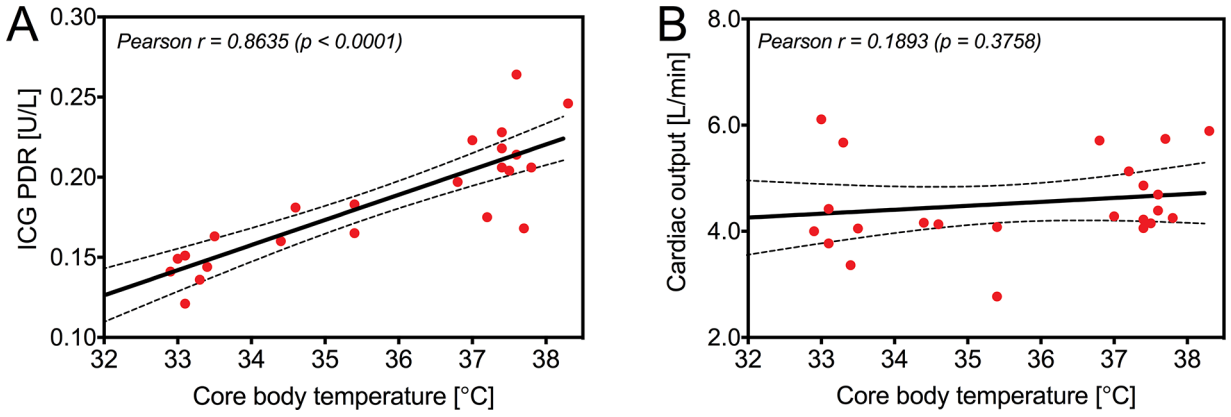

Figure 4. ICG clearance as a function of core body temperature in pigs. Twelve female landrace pigs (mean weight 49 ± 2 kg) were anesthetized (ketamine 5–10 mg/kg/h, sufentanil 5–10 μg/kg/h, isoflurane 0–2%) and subjected to two ICG clearance tests: one at baseline and one after 4 h of passive ambient (20.7 ± 0.6°C) cooling. To that end, 25 mg ICG (PULSION Medical Systems) was administered in 5 mL of 0.9% NaCl via an ear vein catheter. The ICG plasma disappearance rate (PDR) was measured using tail pulse-oximetry. Body temperature, mean arterial pressure and cardiac output were measured with a Swan-Ganz thermodilution catheter placed in the jugular vein. All animal experiments were approved by the institute's animal ethics committee. (A) ICG PDR (*y* axis) plotted as a function of pig core body temperature (*x* axis), which revealed a strong correlation between these parameters (Pearson $r = 0.8635$, $p < 0.0001$; the dashed lines indicate the 95% confidence interval). Corroboratively, linear regression of ICG PDR data yielded a regression equation of $y = -0.376 + 0.016x$ and a goodness of fit (R^2) of 0.7456, which equates to a 0.91-fold increase in ICG clearance for every 1°C drop in core body temperature, thereby approximating the Arrhenius equation. (B) Cardiac output of the pigs remained stable throughout the experiment and was not affected by core body temperature (Pearson $r = 0.1893$, $p = 0.3758$; the dashed lines indicate the 95% confidence interval). Combined with the findings that the pH and mean arterial pressure of the pigs were maintained at physiological levels (data not shown), this indicates that the drop in ICG PDR at lower temperatures is explained by a decrease in basolateral transporter function and is not caused by hypothermia-induced alterations in hepatic perfusion dynamics.

important in light of the role of bile acids as key regulators of liver regeneration. Several reports have shown that an intact enterohepatic circulation is crucial for livers to regenerate after partial hepatectomy (71), which is only possible if both the basolateral and canalicular transporters are fully operational. Although several lines of evidence allude to the involvement of transporter dysfunction at the onset of liver I/R injury, most pathways discussed in this section are based on circumstantial evidence and need to be continued in *in vivo* liver I/R models.

CLINICAL STATUS QUO OF ORGAN COOLING IN LIVER SURGERY

Liver Resection

IHP entails perfusion of the (future remnant) liver with a cold solution during parenchymal transection and thereby aims to reduce I/R injury. The first report on IHP described dual perfusion with chilled Ringer lactate (4°C) through the gastroduodenal artery and the portal vein (5). VIO durations up to 2 h were well tolerated using this technique, enabling the removal of tumors that were otherwise considered not resectable, albeit at the expense of a considerable (10.3%) mortality rate. Consequently, only a small number of technical feasibility studies on IHP in stringently selected cases were published in the following years (7,72,73).

In addition to disappointing clinical outcomes, technical factors have also imposed strict limitations on the use of IHP. First, it is imperative to maintain the core body temperature at >35–36°C during IHP to prevent complications and reduce overall mortality (74,75). The cold perfusate therefore should not enter the systemic circulation, which means the liver must be isolated from the systemic circulation to create a site for safe antegrade perfusate drainage (that is, the vena cava or hepatic veins). These conditions are met through total hepatic vascular exclusion (THVE), a technique that combines VIO with clamping of the infra- and suprahepatic vena cava. During IHP, perfusate drainage occurs via a (cannulated) caval incision. Because THVE imposes a significant burden on hemodynamics, often necessitating a veno-venous bypass, it is only indicated for the removal of large and/or central tumors that impinge on the vena cava and/or hepatic vein(s). Consequently, IHP has been sparsely used.

Despite these technical limitations, several experimental studies have highlighted the therapeutic potential of IHP. The effects of IHP with chilled Ringer lactate solution (4°C) perfused through the hepatic artery were investigated in a pig model of THVE (120 min) (76). At 24 h of reperfusion, improved hepatic microcirculatory perfusion, less endothelial injury and reduced lipid peroxidation were seen after IHP compared to THVE alone (76). In a similar study using 60 min of THVE, survival in the IHP groups (28°C or 20°C liver temperature) was 100% compared with 66% in normothermic THVE controls (30). Moreover, the use of IHP preserved bile production and reduced AST and IL-6 levels. Another study in the same model showed that IHP reduced the increase in PT during reperfusion, attesting to hypothermia-mediated preservation of liver function (77). These beneficial effects of hypothermia on I/R injury renewed clinical interest in IHP.

The first large hypothermia study comprised 20 cases of IHP during THVE that were compared to two THVE control groups (73). IHP was performed when the anticipated duration of THVE was deemed to be >60 min. Hypothermia was established through perfusion with University of Wisconsin solution (4°C) via the portal vein with drainage through a cavotomy. The median (range) liver temperature was 16°C (13°C to 21°C). The results were analyzed after stratification into three treatment groups: IHP (n = 20), THVE <60 min (n = 33) and THVE >60 min (n = 16). Patients treated with IHP had longer VIO durations and underwent more extensive procedures (for example, larger resections and more extrahepatic procedures) compared to the THVE groups, in accordance with the preoperative assessment. However, the mean (± SD) number of complications was 1.2 (± 0.9), 2.6 (± 1.8) and 0.8 (± 1.1) after IHP, >60 min THVE and <60 min THVE, respectively, which was lower in the IHP group compared with the >60 min THVE group. The same applied to postoperative AST and ALT levels, although the mean (± SD) VIO duration was longer in the IHP group compared to the >60 min THVE group (101 ± 16 versus 77 ± 20 min). Morbidity and mortality rates were comparable between the IHP and the THVE groups. The same investigators later reported a 90-d mortality rate of 19.7% for 77 patients who underwent IHP (6). IHP duration and liver temperature were not identified as prognostic factors for 90-d mortality in this study, suggesting only short-term beneficial effects of IHP. Together, these studies show the therapeutic value of IHP in acute clinical outcomes after complex and extensive hepatic resections. These results, however, do not warrant implementation of hypothermia during all major liver resections, since the invasiveness of these techniques does not always outweigh the clinical benefits.

In addition, two recent reports aimed to broaden the use of IHP by circumventing the need for THVE. The first study reported a procedure in which the hepatic veins were clamped and incised, thereby enabling antegrade perfusate drainage without caval clamping (78). Nevertheless, this technique still requires invasive maneuvers such as portocaval shunting. The second study avoided the difficulties of antegrade drainage by enabling the use of IHP without disrupting the hepatic veins or vena cava (7). The procedure was applied during right-sided resections in which Ringer lactate solution (4°C) was perfused through the cut end of the right hepatic artery during VIO with concomitant clamping of the middle/left hepatic vein(s). Retrograde perfusate outflow occurred through the cut end of the right portal vein. A feasibility study in five patients with a median VIO time of 50 min

and a liver temperature of 28°C showed that functional and volumetric liver regeneration was early after IHP and possibly improved compared with a case-matched retrospective control group. A randomized controlled trial comparing this technique with intermittent VIO has recently completed patient recruitment (NCT01499979) and may prompt more widespread implementation of IHP during liver surgery.

Liver Transplantation

The persistent shortage of donor organs has fueled the search for novel preservation strategies that increase the availability of transplantable liver grafts. The current gold standard to preserve liver grafts is static cold storage (SCS), whereby the harvested liver is washed out and stored for a maximum of 15–18 h using a cold (4°C) preservation solution before transplantation. SCS, however, is not optimal, especially for compromised grafts such as those obtained by donation after cardiac death (DCD). Although SCS does reduce liver metabolism to around 5% of baseline, it does not stop anaerobic metabolism and concomitant organ acidification (79). Moreover, SCS does not allow graft viability assessment and has potentially harmful effects on endothelial cells and biliary epithelium.

Although most endothelial cells remain viable during the preservation period, they do undergo morphological changes as a result of proteolytic modification of the extracellular matrix (80). SCS appears to sensitize endothelial cells to reperfusion injury, since most cells die shortly after reperfusion by mechanisms that are still poorly understood (80), but could include injury induced by activated KCs (81) or induction of apoptosis by adherent platelets (82,83). The lack of perfusion during SCS results in collapse of the endothelial glycocalyx (84,85), which promotes I/R injury by facilitating endothelial activation and leukocyte adherence (86). Maintaining microvascular perfusion (see below) during preservation therefore might reduce endothelial cell injury, provided that low (portal) perfusion pressures are used to prevent sheer-induced endothelial injury (87). It should be noted that endothelial preservation is difficult during prolonged preservation times (84). In biliary epithelium, ATP depletion during SCS results in detachment of cholangiocytes from the basal membrane (85). This type of injury is a feared complication after liver transplantation and, together with stromal necrosis, may lead to the formation of posttransplant biliary strictures (88).

To overcome these drawbacks, machine perfusion (MP) techniques have emerged as an alternative to SCS. MP entails the perfusion of harvested grafts with a preservation solution with the purpose of reducing preservation injury by providing metabolic support and mimicking *in vivo* perfusion dynamics while concomitantly removing toxic waste products. Because MP systems are also equipped with temperature control units, MP can be performed at hypothermic (HMP, 4°C), subnormothermic (SNMP, 20° to 30°C) or normothermic (NMP, 37°C) temperatures, which all have a specific set of pros and cons.

In NMP, the risks of hypothermia are avoided by preserving the graft at 37°C. Although graft viability can be assessed in all machine perfusion setups, NMP theoretically offers the best context to sample the perfusate for injury markers (for example, ALT, lactate), since liver metabolism is maintained at a physiological level and additional injury by warm reperfusion is avoided. In a pig model of liver preservation, 10 h of NMP proved superior to SCS, as determined by liver injury parameters (AST release, bile production) as well as liver histology after 24 h of reperfusion (89). In a similar experimental model, SCS elicited severe biliary necrosis, compared with only mild biliary damage after NMP (90). In addition, cholangiocyte proliferation was observed after NMP, indicating that NMP might be able to reduce biliary complications after transplantation by securing adequate cholangiocyte oxygenation during preservation. NMP was successfully used to revive discarded human liver grafts on the basis of improved liver histology, increased bile production and biochemical parameters (ALT release, lactate production) (91). It should be noted that these grafts were not transplanted, meaning that all discussed NMP data were obtained in an *ex vivo* setting. Despite these encouraging results, the sustained metabolic rate during NMP requires oxygenation of the perfusate to provide adequate metabolic support and prevent warm ischemia. This need has driven research into the use of oxygen carriers such as red blood cells, which is complicated and expensive (92). Reducing the oxygen demand through hypothermia may hence eliminate the critical mismatch between oxygen supply and demand.

HMP provides a "middle ground" between NMP and SCS and currently is the established preservation technique for kidney grafts (93). HMP combines the benefits of reduced metabolism by hypothermia (usually 4°C) with the ability to provide oxygen (without oxygen carriers) and remove toxic metabolites by continuous liver perfusion. HMP has shown promise in several animal models of liver preservation (94,95). When compared with SCS in a rat model of orthotopic liver transplantation, HMP resulted in less biliary injury and reduced I/R injury, as demonstrated by a drop in inflammatory cell influx and ALT release (94,95). The reduction in biliary injury after HMP probably results from the combination of perfusate oxygenation and hypothermia, which in concert neutralize the main trigger of posttransplant biliary injury (that is, ATP depletion). A study using the same rat model of liver transplantation more recently revealed that HMP is also superior to NMP in its ability to prevent ROS formation and immune activity after transplantation (92). Hypothermia during (oxygenated) MP reduces the ischemic disruption of mitochondrial metabolism, thereby reducing the extent of ROS formation and consequent liver injury during reperfusion (96). As a result, attenuated DAMP release prevents

KC activation and limits leukocyte-mediated parenchymal injury (97).

The first clinical series comparing HMP with SCS showed a significant reduction in primary graft dysfunction in the HMP group (98). It should be noted that non-oxygenated HMP was used in the latter study, which complicates direct comparison with actively oxygenated HMP studies. In a more recent series of eight patients who had received marginal (donation after cardiac death) grafts resuscitated with oxygenated HMP (1–2 h before implantation), the clinical outcomes were comparable to eight matched control patients who received grafts from donation after brain death (99), which are of superior quality because these grafts do not suffer from extended periods of warm ischemia. These promising results were further corroborated with marginal liver grafts. In a series of 31 patients, HMP and transplantation of normally declined liver grafts performed equally to matched control patients who received "regular" livers preserved using SCS in terms of early graft dysfunction rates, biliary complications and 1-year survival (100). These results could extend the use of marginal liver grafts and warrant additional clinical trials that explore the benefits of (oxygenated) HMP.

HMP does compromise grafts by cold-induced damage to endothelial and biliary epithelial cells. In line with I/R research (25,29), hypothermia (4°C) might not confer additional protection when compared with subnormothermic temperatures (20° to 30°C), whereas subnormothermic temperatures could circumvent the drawbacks associated with deep hypothermia (101). In a rat model of liver transplantation, SNMP at 20°C improved the hepatocellular energy status and reduced ROS formation during reperfusion compared to SCS (101). In this model, SNMP was similar to HMP in terms of AST release. However, γ-glutamyl transferase (γGT) levels were lower in the SNMP group, implicating a reduction in biliary injury. Furthermore, SNMP reduced ROS formation compared to HMP and improved parenchymal energy (that is, ATP) status. These findings were extended to a feasibility study using discarded human livers, in which SNMP at 21°C effectively preserved liver function during the preservation period (102). The latter was reflected by an increase in hepatic ATP, urea and albumin levels throughout the SNMP procedure as well as by the histological profiles before and after the perfusion period (102). Clinical studies on SNMP are currently lacking, and future investigations could provide additional insight into the potential benefits of SNMP over other perfusion techniques.

Drawing firm conclusions from the currently available experimental and clinical MP data remains challenging. The applied temperature, the perfusion settings, the perfusate composition, the extent of oxygenation and the reperfusion method all vary between studies, and each variable could influence the outcome parameters. Furthermore, most experimental studies use continuous preservation, while most clinical reports only apply reconditioning after SCS because of logistic reasons. Lastly, direct (clinical) comparison of different MP techniques is not yet available.

These considerations notwithstanding, SCS still is the standard strategy to preserve liver grafts, despite the excellent results of MP techniques in the experimental and clinical setting. Although SCS provides a simple, affordable and portable method, MP could expand the donor pool by extending tolerable preservation times and rendering marginal grafts viable for transplantation. As various MP regimes have already shown a benefit over SCS, MP will likely replace SCS as the gold standard liver preservation technique in the near future. With respect to the preferred MP modality, HMP appears to be the safest preservation method currently available, at least until SNMP regimens have been optimized and results from ongoing NMP trials and trials directly comparing MP techniques become available (103).

CONCLUSION

The consequences of I/R injury still impose time limitations on liver resection and transplantation procedures. The metabolic response of hepatocyte mitochondria during ischemia lies at the root of this problem, since it is directly linked to several hallmarks of hepatic I/R injury, including mitochondrial ROS production, activation of the innate immune system, the onset of postoperative hyperglycemia and hepatocyte transporter dysfunction. Reducing the metabolic rate of ischemic hepatocytes through hypothermia favorably modulates these processes, which not only explains how hypothermia reduces I/R injury, but also identifies a novel set of outcome parameters that can be used to evaluate the therapeutic efficacy of hypothermia in experimental as well as clinical hepatic I/R research. Expanding the use of IHP during liver resection as well as finding the optimal MP temperature remain key challenges. Based on current data, therapeutic hypothermia may especially benefit patients with a reduced ischemic tolerance due to steatosis or other metabolic disorders.

ACKNOWLEDGMENTS

The authors thank Inge Kos from the medical illustration service for some of the artwork. RF van Golen was supported by a PhD scholarship and the Young Talent Fund, both from the Academic Medical Center in Amsterdam. M Heger was supported by grants from the Dutch Anti-Cancer Foundation (Stichting Nationaal Fonds Tegen Kanker) in Amsterdam, the Phospholipid Research Center in Heidelberg, the Nijbakker-Morra Foundation in Leiden, and Stichting Technologische Wetenschap (STW).

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Farges O, Goutte N, Bendersky N, Falissard B, Group AC-FHS. (2012) Incidence and risks of liver resection: an all-inclusive French nationwide study. *Ann. Surg.* 256:697–704.
2. Breitenstein S, *et al.* (2010) Novel and simple preoperative score predicting complications after liver resection in noncirrhotic patients. *Ann. Surg.* 252:726–34.
3. Schiergens TS, *et al.* (2014) Liver resection in the elderly: significance of comorbidities and blood loss. *J. Gastrointest. Surg.* 18:1161–70.
4. van Golen RF, Reiniers MJ, van Gulik TM, Heger M. (2015) Organ cooling in liver transplantation and resection: how low should we go? *Hepatology.* 61:395–9.
5. Fortner JG, *et al.* (1974) Major hepatic resection using vascular isolation and hypothermic perfusion. *Ann. Surg.* 180:644–52.
6. Azoulay D, *et al.* (2015) Complex liver resection using standard total vascular exclusion, venovenous bypass, and in situ hypothermic portal perfusion: an audit of 77 consecutive cases. *Ann. Surg.* 262:93–104.
7. Reiniers MJ, *et al.* (2014) In situ hypothermic perfusion with retrograde outflow during right hemihepatectomy: first experiences with a new technique. *J. Am. Coll. Surg.* 218:e7–16.
8. van Golen RF, van Gulik TM, Heger M. (2012) The sterile immune response during hepatic ischemia/reperfusion. *Cytokine Growth Factor Rev.* 23:69–84.
9. van Golen RF, van Gulik TM, Heger M. (2012) Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury. *Free Radic. Biol. Med.* 52:1382–1402.
10. Kloek JJ, *et al.* (2012) Cholestasis is associated with hepatic microvascular dysfunction and aberrant energy metabolism before and during ischemia-reperfusion. *Antioxid. Redox Signal.* 17:1109–23.
11. Chouchani ET, *et al.* (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature.* 515:431–5.
12. Murphy MP. (2009) How mitochondria produce reactive oxygen species. *Biochem. J.* 417:1–13.
13. Radi R, Rodriguez M, Castro L, Telleri R. (1994) Inhibition of mitochondrial electron transport by peroxynitrite. *Arch. Biochem. Biophys.* 308:89–95.
14. Kim JS, Wang JH, Lemasters JJ. (2012) Mitochondrial permeability transition in rat hepatocytes after anoxia/reoxygenation: role of Ca2+-dependent mitochondrial formation of reactive oxygen species. *Am. J. Physiol.* 302:G723–31.
15. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S. (2002) Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. U. S. A.* 99:1259–63.
16. Lemasters JJ, Theruvath TP, Zhong Z, Nieminen AL. (2009) Mitochondrial calcium and the permeability transition in cell death. *Biochim. Biophys. Acta.* 1787:1395–401.
17. Nieminen AL, Byrne AM, Herman B, Lemasters JJ. (1997) Mitochondrial permeability transition in hepatocytes induced by t-BuOOH: NAD(P)H and reactive oxygen species. *Am. J. Physiol.* 272: C1286–1294.
18. van Golen RF, Reiniers MJ, Olthof PB, van Gulik TM, Heger M. (2013) Sterile inflammation in hepatic ischemia/reperfusion injury: present concepts and potential therapeutics. *J. Gastroenterol. Hepatol.* 28:394–400.
19. de Graaf W, *et al.* (2012) Quantitative assessment of liver function after ischemia-reperfusion injury and partial hepatectomy in rats. *J. Surg. Res.* 172: 85–94.
20. Lock JF, *et al.* (2009) The costs of postoperative liver failure and the economic impact of liver function capacity after extended liver resection: a single-center experience. *Langenbecks Arch. Surg.* 394:1047–56.
21. Thorniley MS, Simpkin S, Fuller B, Jenabzadeh MZ, Green CJ. (1995) Monitoring of surface mitochondrial NADH levels as an indication of ischemia during liver isograft transplantation. *Hepatology.* 21:1602–9.
22. Caraceni P, Ryu HS, van Thiel DH, Borle AB. (1995) Source of oxygen free radicals produced by rat hepatocytes during postanoxic reoxygenation. *Biochim. Biophys. Acta.* 1268:249–54.
23. Barron JT, Gu L, Parrillo JE. (1998) Malate-aspartate shuttle, cytoplasmic NADH redox potential, and energetics in vascular smooth muscle. *J. Mol. Cell. Cardiol.* 30:1571–9.
24. Zhou L, Stanley WC, Saidel GM, Yu X, Cabrera ME. (2005) Regulation of lactate production at the onset of ischaemia is independent of mitochondrial NADH/NAD+: insights from in silico studies. *J. Physiol.* 569:925–37.
25. Behrends M, *et al.* (2006) Mild hypothermia reduces the inflammatory response and hepatic ischemia/reperfusion injury in rats. *Liver Int.* 26:734–741.
26. Niemann CU, *et al.* (2006) Mild hypothermia protects obese rats from fulminant hepatic necrosis induced by ischemia-reperfusion. *Surgery.* 140:404–12.
27. Khandoga A, *et al.* (2003) Impact of intraischemic temperature on oxidative stress during hepatic reperfusion. *Free Radic. Biol. Med.* 35:901–9.
28. Harrison R. (2002) Structure and function of xanthine oxidoreductase: where are we now? *Free Radic. Biol. Med.* 33:774–97.
29. Biberthaler P, *et al.* (2001) The influence of organ temperature on hepatic ischemia-reperfusion injury: a systematic analysis. *Transplantation.* 72:1486–90.
30. Dinant S, van Veen SQ, Roseboom HJ, van Vliet AK, van Gulik TM. (2006) Liver protection by hypothermic perfusion at different temperatures during total vascular exclusion. *Liver Int.* 26:486–93.
31. van Golen RF, Reiniers MJ, Heger M, Verheij J. (2015) Solutions to the discrepancies in the extent of liver damage following ischemia/reperfusion in standard mouse models. *J. Hepatol.* 62:975–7.
32. Churchill TA, Cheetham KM, Fuller BJ. (1994) Glycolysis and energy metabolism in rat liver during warm and cold ischemia: evidence of an activation of the regulatory enzyme phosphofructokinase. *Cryobiology.* 31:441–52.
33. Reiniers MJ, van Golen RF, van Gulik TM, Heger M. (2014) Reactive oxygen and nitrogen species in steatotic hepatocytes: a molecular perspective on the pathophysiology of ischemia-reperfusion injury in the fatty liver. *Antioxid. Redox Signal.* 21:1119–42.
34. Moon KH, *et al.* (2008) Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion. *Gastroenterology.* 135:1344–57.
35. Jaeschke H, Lemasters JJ. (2003) Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. *Gastroenterology.* 125:1246–57.
36. Takahashi K, Morikawa S, Inubushi T, Nosaka S. (2004) Protective effects of moderate hypothermia on phosphoenergetic metabolism in rat liver during gradual hypoxia studied by in vivo 31P nuclear magnetic resonance spectroscopy. *J. Surg. Res.* 117:323–8.
37. Peralta C, *et al.* (2000) Hepatic preconditioning preserves energy metabolism during sustained ischemia. *Am. J. Physiol. Gastrointest. Liver Physiol.* 279:G163–71.
38. Dutta A, *et al.* (2009) Impairment of mitochondrial beta-oxidation in rats under cold-hypoxic environment. *Int. J. Biometeorol.* 53:397–407.
39. Johannigman JA, Johnson DJ, Roettger R. (1992) The effect of hypothermia on liver adenosine triphosphate (ATP) recovery following combined shock and ischemia. *J. Trauma.* 32:190–5.
40. Niemann CU, *et al.* (2010) Short passive cooling protects rats during hepatectomy by inducing heat shock proteins and limiting the induction of pro-inflammatory cytokines. *J. Surg. Res.* 158:43–52.
41. van Golen RF, Reiniers MJ, Verheij J, Heger M. (2015) Solutions to the discrepancies in liver damage profiles following ischemia/reperfusion in standardized mouse models. *J. Hepatol.* 62:975–7.
42. Kato A, Singh S, McLeish KR, Edwards MJ, Lentsch AB. (2002) Mechanisms of hypothermic protection against ischemic liver injury in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 282:G608–16.
43. Seki E, Brenner DA, Karin M. (2012) A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. *Gastroenterology.* 143:307–20.
44. Zwacka RM, *et al.* (1998) Redox gene therapy for ischemia/reperfusion injury of the liver reduces AP1 and NF-kappaB activation. *Nat. Med.* 4:698–704.
45. Kuboki S, *et al.* (2007) Hepatocyte NF-kappaB activation is hepatoprotective during ischemia-reperfusion injury and is augmented by ischemic hypothermia. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G201–7.

46. Shin T, Kuboki S, Lentsch AB. (2008) Roles of nuclear factor-kappaB in postischemic liver. *Hepatol. Res.* 38:429–40.
47. Yenari MA, *et al.* (2005) Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. *Ann. N. Y. Acad. Sci.* 1053:74–83.
48. Kuboki S, *et al.* (2007) Role of heat shock protein 70 in hepatic ischemia-reperfusion injury in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G1141–9.
49. Iimuro Y, *et al.* (1998) NFkappaB prevents apoptosis and liver dysfunction during liver regeneration. *J. Clin. Invest.* 101:802–11.
50. Konig M, Bulik S, Holzhutter HG. (2012) Quantifying the contribution of the liver to glucose homeostasis: a detailed kinetic model of human hepatic glucose metabolism. *PLoS Comput. Biol.* 8:e1002577.
51. Shi Z, *et al.* (2013) Prevalence of stress hyperglycemia among hepatopancreatobiliary postoperative patients. *Int. J. Clin. Exp. Med.* 6:799–803.
52. Huang PY, *et al.* (2015) Correlation of early postoperative blood glucose levels with postoperative complications, hospital costs, and length of hospital stay in patients with gastrointestinal malignancies. *Endocrine.* 48:187–94.
53. Han S, *et al.* (2015) Glycemic responses to intermittent hepatic inflow occlusion in living liver donors. *Liver Transpl.* 21:180–6.
54. Little SA, Jarnagin WR, DeMatteo RP, Blumgart LH, Fong Y. (2002) Diabetes is associated with increased perioperative mortality but equivalent long-term outcome after hepatic resection for colorectal cancer. *J. Gastrointest. Surg.* 6:88–94.
55. van den Berghe G, *et al.* (2001) Intensive insulin therapy in critically ill patients. *N. Engl. J. Med.* 345:1359–67.
56. Durczynski A, *et al.* (2013) Major liver resection results in early exacerbation of insulin resistance, and may be a risk factor of developing overt diabetes in the future. *Surg. Today.* 43:534–8.
57. Leclercq IA, Da Silva Morais A, Schroyen B, Van Hul N, Geerts A. (2007) Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. *J. Hepatol.* 47:142–56.
58. Uehara T, *et al.* (2005) JNK mediates hepatic ischemia reperfusion injury. *J. Hepatol.* 42:850–9.
59. Michael MD, *et al.* (2000) Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol. Cell.* 6:87–97.
60. Kiuchi T, *et al.* (1990) Changes in arterial ketone body ratio in the phase immediately after hepatectomy: prognostic implications. *Arch. Surg.* 125:655–9.
61. Choi S, *et al.* (2005) Mild hypothermia provides significant protection against ischemia/reperfusion injury in livers of obese and lean rats. *Ann. Surg.* 241:470–6.
62. International Transporter Consortium, *et al.* (2010) Membrane transporters in drug development. *Nat. Rev. Drug Discov.* 9:215–36.
63. Huang W, *et al.* (2006) Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science.* 312:233–6.
64. de Graaf W, *et al.* (2011) Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. *J. Hepatol.* 54:738–45.
65. Hoekstra LT, *et al.* (2013) Physiological and biochemical basis of clinical liver function tests: a review. *Ann. Surg.* 257:27–36.
66. Tanaka Y, Chen C, Maher JM, Klaassen CD. (2006) Kupffer cell-mediated downregulation of hepatic transporter expression in rat hepatic ischemia-reperfusion. *Transplantation.* 82:258–66.
67. Tsujimoto T, *et al.* (2013) Effect of oxidative stress on expression and function of human and rat organic anion transporting polypeptides in the liver. *Int. J. Pharm.* 458:262–71.
68. Heijnen BH, Straatsburg IH, Gouma DJ, van Gulik TM. (2003) Decrease in core liver temperature with 10 degrees C by in situ hypothermic perfusion under total hepatic vascular exclusion reduces liver ischemia and reperfusion injury during partial hepatectomy in pigs. *Surgery.* 134:806–17.
69. Roth M, Obaidat A, Hagenbuch B. (2012) OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br. J. Pharmacol.* 165:1260–87.
70. Jonker JW, Stedman CA, Liddle C, Downes M. (2009) Hepatobiliary ABC transporters: physiology, regulation and implications for disease. *Front. Biosci.* 14:4904–20.
71. Naugler WE. (2014) Bile acid flux is necessary for normal liver regeneration. *PLoS One.* 9:e97426.
72. Hoti E, Salloum C, Azoulay D. (2011) Hepatic resection with in situ hypothermic perfusion is superior to other resection techniques. *Dig. Surg.* 28:94–9.
73. Azoulay D, *et al.* (2005) In situ hypothermic perfusion of the liver versus standard total vascular exclusion for complex liver resection. *Ann. Surg.* 241:277–85.
74. Kurz A, Sessler DI, Lenhardt R. (1996) Perioperative normothermia to reduce the incidence of surgical-wound infection and shorten hospitalization: Study of Wound Infection and Temperature Group. *N. Engl. J. Med.* 334:1209–1215.
75. Frank SM, *et al.* (1997) Perioperative maintenance of normothermia reduces the incidence of morbid cardiac events: a randomized clinical trial. *JAMA.* 277:1127–34.
76. Heijnen BH, *et al.* (2003) Effect of in situ hypothermic perfusion on intrahepatic pO2 and reactive oxygen species formation after partial hepatectomy under total hepatic vascular exclusion in pigs. *Liver Int.* 23:19–27.
77. Dinant S, Roseboom HJ, Levi M, van Vliet AK, van Gulik TM. (2009) Hypothermic in situ perfusion of the porcine liver using Celsior or Ringer-lactate solution. *Langenbecks Arch. Surg.* 394:143–50.
78. Azoulay D, *et al.* (2014) Liver resection using total vascular exclusion of the liver preserving the caval flow, in situ hypothermic portal perfusion and temporary porta-caval shunt: a new technique for central tumors. *Hepatobiliary Surg. Nutr.* 3:149–53.
79. Belzer FO, Southard JH. (1988) Principles of solid-organ preservation by cold storage. *Transplantation.* 45:673–6.
80. Clavien PA. (1998) Sinusoidal endothelial cell injury during hepatic preservation and reperfusion. *Hepatology.* 28:281–5.
81. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. (1991) Kupffer cell activation and endothelial cell damage after storage of rat livers: effects of reperfusion. *Hepatology.* 13:83–95.
82. Sindram D, Porte RJ, Hoffman MR, Bentley RC, Clavien PA. (2000) Platelets induce sinusoidal endothelial cell apoptosis upon reperfusion of the cold ischemic rat liver. *Gastroenterology.* 118:183–91.
83. van Golen RF, Stevens KM, Colarusso P, Jaeschke H, Heger M. (2015) Platelet aggregation but not activation and degranulation during the acute post-ischemic reperfusion phase in livers with no underlying disease. *J. Clin. Transl. Res.* 1:107–15.
84. Xu H, Lee CY, Clemens MG, Zhang JX. (2004) Pronlonged hypothermic machine perfusion preserves hepatocellular function but potentiates endothelial cell dysfunction in rat livers. *Transplantation.* 77:1676–82.
85. Brunner SM, *et al.* (2013) Bile duct damage after cold storage of deceased donor livers predicts biliary complications after liver transplantation. *J. Hepatol.* 58:1133–9.
86. van Golen RF, *et al.* (2014) The mechanisms and physiological relevance of glycocalyx degradation in hepatic ischemia/reperfusion injury. *Antioxid. Redox Signal.* 21:1098–118.
87. Schlegel A, Kron P, Dutkowski P. (2015) Hypothermic oxygenated liver perfusion: basic mechanisms and clinical application. *Curr. Transplant. Rep.* 2:52–62.
88. Westerkamp AC, *et al.* (2015) End-ischemic machine perfusion reduces bile duct injury in donation after circulatory death rat donor livers. *Liver Transpl.* 21:1300–11.
89. Nassar A, *et al.* (2015) Ex vivo normothermic machine perfusion is safe, simple, and reliable: results from a large animal model. *Surg. Innov.* 22:61–9.
90. Liu Q, *et al.* (2014) Sanguineous normothermic machine perfusion improves hemodynamics and biliary epithelial regeneration in donation after cardiac death porcine livers. *Liver Transpl.* 20:987–99.
91. op den Dries S, *et al.* (2013) Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. *Am. J. Transplant.* 13:1327–35.

92. Schlegel A, Kron P, Graf R, Dutkowski P, Clavien PA. (2014) Warm vs. cold perfusion techniques to rescue rodent liver grafts. *J. Hepatol.* 61:1267–75.
93. Moers C, *et al*. (2009) Machine perfusion or cold storage in deceased-donor kidney transplantation. *N. Engl. J. Med.* 360:7–19.
94. Schlegel A, Graf R, Clavien PA, Dutkowski P. (2013) Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation. *J. Hepatol.* 59:984–91.
95. Dutkowski P, Furrer K, Tian Y, Graf R, Clavien PA. (2006) Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. *Ann. Surg.* 244:968–76.
96. Graham JA, Guarrera JV. (2014) "Resuscitation" of marginal liver allografts for transplantation with machine perfusion technology. *J. Hepatol.* 61:418–31.
97. Schlegel A, Rougemont O, Graf R, Clavien PA, Dutkowski P. (2013) Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *J. Hepatol.* 58:278–86.
98. Guarrera JV, *et al.* (2010) Hypothermic machine preservation in human liver transplantation: the first clinical series. *Am. J. Transplant.* 10:372–81.
99. Dutkowski P, *et al.* (2014) HOPE for human liver grafts obtained from donors after cardiac death. *J. Hepatol.* 60:765–72.
100. Guarrera JV, *et al.* (2015) Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. *Am. J. Transplant.* 15:161–9.
101. Vairetti M, *et al.* (2009) Subnormothermic machine perfusion protects steatotic livers against preservation injury: a potential for donor pool increase? *Liver Transpl.* 15:20–9.
102. Bruinsma BG, *et al.* (2014) Subnormothermic machine perfusion for ex vivo preservation and recovery of the human liver for transplantation. *Am. J. Transplant.* 14:1400–9.
103. Guarrera JV. (2014) Donation: where are our opportunities for expansion? *Liver Transp.* 20(S2):S2–4.

Cite this article as: Olthof PB, *et al*. (2015) Protective mechanisms of hypothermia in liver surgery and transplantation. *Mol. Med*. 21:833–46.