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## Chapter 4

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# Expanding the Donor Pool in Liver Transplantation: Influence of Ischemia-Reperfusion

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## Abstract

Improvements in surgical techniques, immunosuppression, and patient management have led to the optimization of liver transplantation outcomes. However, the waiting list for liver transplantation is increasing at a greater pace. The large imbalance between the growing pool of potential liver transplant recipients and the scarcity of donor organs has fueled efforts to maximize existing donors and identify new sources.

To expand the potential donor pool, clinical, and organ procurement agencies are continually modifying the criteria of an acceptable liver donor and are looking to marginal or expanded donors to meet the waiting list demands. This book chapter will be focused on the current state of liver transplantation using grafts from extended criteria donors (elderly donors, steatotic donors, donors with malignancies, donors with viral hepatitis) and from donation after cardiac death (non-heart beating donors), as well as the use of partial grafts (split grafts and living-donor liver transplantation) and other suboptimal donors (donors with hypernatraemia, infections, hypotension and inotropic support). Overall, broadened criteria for acceptable donor livers appear to lessen graft survival rates somewhat compared with rates for ideal donor organs.

Donors are generally considered marginal if there is a risk of initial poor function or primary non-function. The present book chapter will discuss the factors defining marginality of a graft, the pathophysiology of the marginal donor, and the issues faced by transplant units in making the decision to use such a graft; along with strategies for minimizing the ischemia-reperfusion injury experienced by the organs. We will show the

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experimental models used to study the complexity of hepatic ischemia-reperfusion injury in marginal donors. Data reported in animal models and the different strengths and limitations of the different experimental models will be also discussed. This is a valuable tool for discovering novel therapeutic targets and drugs. New surgical and pharmacological strategies for improving the function of the marginal/expanded donor liver also will be reviewed. This would be of clinical interest to reduce their prevalence and improve their management. As we will discuss in the book chapter, at this time the management of marginal donors is empirical, being currently based on clinical practical experience. We will show that further experimental research is needed to identify better tests for evaluating donor organs, provide longer-term follow-up of recipients of higher-risk organs, and develop alternative means to fill the donor-organ shortfall.

## Introduction

Liver transplantation has evolved as the therapy of choice for patients with end-stage liver disease. Improvements in surgical techniques, immunosuppression, and patient management have led to the optimization of liver transplantation outcomes. However, the waiting list for liver transplantation is increasing at a greater pace and each year a greater number of patients die while awaiting donor organs [1]. The large imbalance between the growing pool of potential liver transplant recipients and the scarcity of donor organs has fueled efforts to maximize existing donors and identify new sources.

A major challenge for the transplant community is to develop strategies to close the gap between the number of patients in need of a transplant and the number of available organs. Scientists, clinical and organ procurement agencies are expanding the donor pool through two mechanisms. The first mechanism is using organs that were previously thought to be associated with an high risk of primary nonfunction (PNF) or initial poor function (IPF), so called extended criteria donors or marginal livers [2] (i.e. donors with steatosis, with malignancies, with viral infections, older or elderly donors, donors after cardiac death and others). These marginal livers considered unacceptable for transplantation, are now being transplanted, but the main difficulty is in defining the criteria that can be extended, because this criteria vary between centers and regions. The second way to expand the donor pool is through advances in medical practice, particularly surgical techniques including split liver transplantation (SLT) and living donor liver transplantation (LDLT), all of these will be mentioned along this book chapter. Although the organs from marginal donors may not be optimal, the high death rate on the waiting lists produced a stark choice between dying without a liver or proceeding with a liver that was perhaps not ideal [1]. It is known that the marginal grafts exhibit poor tolerance to Ischemia-Reperfusion (I/R). I/R injury is an important cause of liver damage occurring during surgical procedures including hepatic resections and liver transplantation (LT) [3]. Also, I/R injury is the underlying cause of graft dysfunction in marginal organs [1]. Moreover, I/R affect negatively the process of liver regeneration in surgical conditions including hepatic resections and small-for size LT [3].

The present book chapter will discuss the factors defining marginality of a graft, the pathophysiology of the marginal donor, and the issues faced by transplant units in making the decision to use such a graft; along with strategies for minimizing the I/R injury experienced by the livers submitted to transplantation. We will show the experimental models used to study the complexity of hepatic I/R injury in marginal donors and the new surgical and

pharmacological strategies for manipulating and improving the function of the marginal organs. This would be of clinical interest to reduce their prevalence and improve their management. The ongoing effort to expand the pool of usable liver grafts has made it clear that a better understanding of the mechanisms of I/R injury and other consequences of using marginal grafts are critical to improving results with these grafts.

## Marginal Livers for Transplantation

A marginal liver could be defined as an organ with an increased risk of IPF or PNF that may cause greater risks of morbidity or mortality in the recipient. However, there is no consensus, about the specific factors that define a graft as marginal or about which factors or combinations thereof should exclude the graft from use because of unacceptable risk to the recipient [4]. However, some of the marginal liver donor criteria used are as follows: Obesity (weight >100 Kg or BMI >27), Age >50 years; Macrovesicular steatosis >50%; Intensive care unit stay >4 days; Cold ischemia time >14 h; Prolonged hypotensive episodes of >1 h, and <60 mm Hg with high inotropic support (dopamine >14 µg/kg per minute); Hypernatremia (peak serum sodium >155 mEq/L); Viral infections; Sepsis and alcoholism; Extrahepatic neoplasia; Gender mismatch or Non-heart beating donors (NHBD) [5].

The severity of the resulting liver dysfunction is also determined in part by the degree of hepatic injury that occurs as a consequence of local and systemic haemodynamic changes in response to brain death, liver retrieval and implantation. These factors crucially influence in graft viability [6].

Broadly there are two categories of marginal livers [4]. Firstly there are livers which carry a high risk of technical complications and impaired function (i.e. steatotic donors, NHBD, elderly donors, split livers, and donors with high inotropic requirement). Secondly, grafts will be considered marginal if they carry a risk of transmission infection or malignancy to the recipient (i.e. donor with viral infections or donors with malignancy) (Table 1).

**Table 1. Marginal liver types**

Marginal Liver Types	
Marginal Graft	Steatotic donors
	Elderly or older donors
	Non-heart beating donors
	Donors with hypernatraemia
	Donors with hypotension
	Donors with viral infections
	Donors with malignancies
	Donors with infections
Technical Variant Graft	Split-liver transplantation
	Living-donor liver transplantation

Despite numerous retrospective studies, the impact of each donor variable on graft function and recipient survival is still under investigation because the contradictory results. Some investigators have indicated comparable results regarding graft function and patient

survival after transplantation of marginal donors versus standard grafts, but most reports support a clear correlation between graft quality and post-transplant outcome. New concepts, especially the extended criteria donors scoring system by Cameron et al., [7] and the donor risk index (DRI) by Feng et al., [8] have allowed a more integral and quantitative assessment of the impact of extended donor criteria on post-transplant mortality and the risk of graft failure. According to Cameron et al., [7] a donor older than 55 years, donor hospital stay more than 5 days, cold ischemia time more than 10 h, and warm ischemia time more than 40 minutes were identified as significant criteria with regard to recipient mortality and were assigned one score point each.

Feng et al., [8] analyzed 20,000 transplants from the Scientific Registry of Transplant Recipients (SRTR) database and developed a DRI, which is calculated from seven donor and two transplant variables that were found to be independently associated with an increased risk of graft failure. These included donor older than 40 years, donor height, donation after cardiac death, split/partial grafts, cerebrovascular accident or other cause of death (except trauma, stroke, or anoxia), cold ischemia time, and organ sharing outside the local donor service area. Although a conclusive statement on the impact of graft steatosis could not be made due to incomplete data in the registry, the analysis of Feng et al., [8] highlights the relevant donor risk factors and supports a clear correlation between organ quality and post-transplant outcome.

Actually, the use and acceptance of marginal livers varies between different transplant centers [9], therefore the decision to transplant a specific organ depends on the judgment of the transplant surgeon and consideration of the specific recipients, but even the consequence of using marginal grafts in a future, remains unclear.

## **Types of Marginal Livers**

### **Steatotic Donors**

Hepatic steatosis is defined as lipid accumulation in hepatocytes. Is frequent in cadaveric organ retrievals and live donors, and has been reported in 9% to 26% of donors [10-12]. Given the steady increase in the mean age of cadaver donors and the overall increase in the prevalence of obesity it is expected a further increase in the prevalence of steatosis in both cadaveric and living donors [13]. This represents a large potential pool of donors. The potential use of steatotic livers for transplant, one of the most common types of organs from marginal donors, has become a major focus of investigation. However the clinical problem is still unresolved since steatotic livers are more susceptible to I/R injury and, when used, have poorer outcome than non-steatotic livers. Indeed, the use of steatotic liver for transplantation has been associated with increased incidence of PNF [5, 10, 14] and IPF [15]. Moreover, so nearly one third of all donated livers are discarded because their pathological conditions thus accentuating the problem in the shortage of organs [16]. Therefore, minimizing the adverse effects of I/R injury could improve outcomes in steatotic liver surgery, increase the number both of suitable transplantation grafts and of patients who successfully recover from LT.

Some early studies have shown that graft steatosis is the most important variable in multivariate analysis of factors determining graft function after transplantation [17].

However, steatotic livers can be transplanted safely with good results for long term organ survival especially if other contraindications for their use are absent [13].

The causes of hepatic steatosis are varied and include obesity, older age, alcoholism, diabetes mellitus, hyperlipidemia and postmortem nutritional changes [1]. Histologic patterns show there are two forms of steatosis encountered in liver grafts. 1) Macrovesicular steatosis; in which the fat vacuoles occupy most of the hepatocytes cytoplasm and displace the nucleus peripherally, and considered a more dangerous lesion. 2) Microvesicular steatosis, where the vacuoles are smaller and have a centrilobular distribution, which is commonly found in pathological conditions associated with mitochondrial injury such as some metabolic disorders, is largely reversible and does not tend to cause harmful consequences post-transplant [18]. Severity of steatosis is traditionally graded as mild <30%, moderate 30–60%, and severe >60%. It has been shown that a scoring system that includes degree of steatosis and donor age correlates well with the outcome of fatty livers [19].

The transplantation outcomes are not affected by hepatic microsteatosis, regardless of the severity and have been reported adequate function of livers [20]. In addition, grafts with mild macrosteatosis (<30%) can be safely used, assuming there are no other donor or recipient risk factors, because these livers show similar results to nonsteatotic grafts [21]. Donor livers with severe macrosteatosis (>60%) do have a significant risk of graft failure and should not be used for transplantation, unless there is an urgent situation requiring them to be used as a bridge [11]. The use of grafts with moderate steatosis (>30% and <60%) is controversial, because these may impose a relative risk on post-transplant outcomes. Previous reports have shown an increased incidence of PNF after LT from donors with moderate steatosis compared with nonsteatotic livers (13% vs 3%) [22].

In the transplant setting, a method for determining and measuring the extent of steatosis remains imprecise and inconsistently reported. In particular, the distinction between macrovesicular and microvesicular steatosis is often cited as important, but the precise definition of these, the assessment macroscopically and microscopic and their relative quantification depends on the histological technique and the experience of the interpreting pathologist [23]. On gross examination of the liver, fatty livers are often yellow in color and contain blunted edges, in contrast to the more normal salmon color and sharp borders. However, for the moment microscopic assessment remains the “gold standard” for the diagnosis and quantitation of steatosis [4]. Liver biopsy of the donor liver and frozen section is the preferred method because of time constraints between graft retrieval and transplantation [24] and is considered to be mandatory in special settings.

Recent studies, have shown that ultrasonography, computer tomography and magnetic resonance all display a reasonably good specificity for the diagnosis of steatosis, but also have an unacceptably low sensitivity compared to histology, with the only exception of cases of massive steatosis. Unfortunately, current imaging methods are inaccurate and inadequate in the quantitation of liver steatosis and do not distinguish clearly between the microvesicular and the macrovesicular types [25]. Other tools like, biomechanical impedance and transient elastography (Fibroscan) have been shown to predict steatosis/fibrosis, and the use may be extended in assessment of the donor liver [26]. With increasing prevalence of steatosis in the donor population, more surrogate markers of organ quality are needed.

## Elderly or Older Donors

Donor age steadily increased over recent decades. In 1994, only 20% of deceased donors were 50 years or older. This percentage increased by more than 150% in the year 2004 [27]. Initially donor age >50 years was once considered a contraindication to liver donation because it was thought to be associated with poor graft outcomes, although some studies suggested that older than 50 years without additional risk factors have similar outcomes compared to younger donors [28, 29]. Therefore, given these later results, age itself should not be a contraindication to liver donation. However, Busquets et al., [30] reported that liver grafts from donors >70 years of age had a relative risk of 1.4 and 1.7 for long-term graft failure and mortality, respectively. More recent studies using the large databases of either SRTR/ United Network for Organ Sharing (UNOS) or European Liver Transplantation Registry (ELTR) clearly identified donor age as an important risk factor for poor outcome after LT [31].

In contrast to other organs, the liver may be more immune to senescence, particularly in the otherwise healthy person. This is possibly because of the liver's large functional reserve, regenerative capacity, and dual blood supply, which exceeds its metabolic needs [32, 33]. On the other hand, older donor livers tend to be smaller (in weight and volume) and darker-colored, and may have developed fibrous thickening of the capsule [34] than younger livers, as well as blood flow are reduced with aging [35]. Whether these morphologic changes impact on organ function after transplantation remains to be elucidated. It has been shown that older donor livers are more susceptible to endothelial cell injury from cold ischemia and show decreased ATP synthesis after reperfusion, which may influence the decreased regenerative capacity [36] and decreased synthetic function [37].

Some factors including steatosis or prolonged ischemia could contribute to the poor post-transplantation outcomes from elderly donors [38]. Attention should be paid to the possible effects of atherosclerosis on arterial vessels. Calcified plaques on the hepatic artery might result in severe complications [39]. Elderly donor also appears to have an additive adverse effect on liver recipients with hepatitis C virus (HCV). [40]. Also donor age may be important in recipients with primary biliary cirrhosis as this can adversely affect their outcome [41]. Transmission of malignancy is another consideration with aged donors because of the higher incidence of unrecognized malignancies in the elderly [42].

## Donation after Cardiac Death

This group of organs forms the basis for most organ donation and in the last few years has seen a considerable renewal of interest in NHBD also referred to as donation after cardiac death as a potential to increase the pool of available organs [4]. The potential contribution of NHBD is difficult to estimate, however it has been reported to comprise between 4% and 20% of transplanted grafts among centers with high rates of use [43].

NHBD are divided into controlled and uncontrolled donation based on Maastricht classification in order to underline differences in clinical practice and graft outcome. Controlled donations occurs with a circulatory arrest after planned withdrawal of life support equipment, most often in an intensive care unit in a controlled environment with a donor surgical team available. In uncontrolled donation, the donor death occurs completely

unplanned, outside the hospital or in the emergency room following an unplanned cardiac arrest with unsuccessful attempt of resuscitation [44]. In controlled NHBD, warm ischemia time can be accurately assessed, cold ischemia can be minimized, therefore are comparatively far less prone to ischemic damage and tend to offer superior post-transplant function [45]. This was not the case for uncontrolled NHBD since in this clinical situation, the organs suffer severe ischemic insult. Liver allograft survival from uncontrolled NHBDs has been poor (17% to 41%) [46].

Ischemic time has been shown to be extremely important when NHBD are considered [47]. If warm ischemic time is restricted to below 30 minutes and cold ischemia time less than 10 h, graft survival rate in the NHBD group has been found to be 81% and 67% at 1 and 3 years respectively, which is not significantly different from recipients of brain dead donors [48]. Results from uncontrolled NHBD were less good, being graft survival at 2 years of 55%. The use of uncontrolled NHBD livers was also associated with significantly higher incidence of PNF, IPF and biliary complications [49].

It is likely that further refinements in patient selection, operative technique and preservation solutions will improve the results and utility of NHBD and potentially expand the donor pool by 20–30% [43].

### Donors with Hypernatraemia

Hypernatremia was shown to be one of five variables with prognostic value in predicting graft survival after transplantation [50]. Some studies have suggested that donors with hypernatraemia can affect graft function and increase the risk of graft loss [51]. The mechanism for the deleterious effect of elevated donor sodium on graft function is thought to be a result of cell swelling, increased osmolality and exacerbation of reperfusion-mediated injury [1]. The cause of hypernatraemia could be related to derangement of fluid balance and diabetes insipidus in potential donors [51]. In a study investigating the peak donor sodium level and the corrected sodium level at the time of retrieval, it was found that hypernatraemia (sodium >155 mEq/l) was associated with 18.5% rate of PNF compared with 3.4% in the normal sodium group. With the correction of hypernatraemia before procurement, this rise in the PNF was no longer found [51]. Another pilot study at University of California examined the effects of infusing 5% dextrose (D<sub>5</sub>W) in water through the inferior mesenteric vein before harvesting the organ if the donor sodium level was greater than 160 mEq/L. In the 17 donors that received the D<sub>5</sub>W to decrease hypernatremia, the rates of recipient DNF/PNF were 0% compared with a group of historical controls that experienced a 60% incident of delayed non-function/PNF [1].

### Donors with Hypotension and Inotropic Support

Previous UNOS data have shown that donor organs subjected to prolonged hypotension have no significant increase in post-transplantation graft loss. However, graft loss was increased in liver transplant recipients when donors received norepinephrine [52]. In other studies, dopamine dose >10 µg/kg/min [50] or 6 µg/kg/min [53] had a significant effect on

early graft function. However, other factors such as age and fat content may modify these effects in either direction.

Briceño et al., [54] reported that unstable donors with high doses of inotropic drugs have an increase in severe preservation damage rate, and trends to normalize hemodynamic status in brain-death donors did not correct liver dysfunction. Probably, time-dependent administrations of high-dose dopamine and epinephrine have a harmful effect on liver function.

## Donors with Viral Infections

Potential donors with positive viral infections should not be completely ruled out from the donor pool [4]. Viral infections such as hepatitis B, hepatitis C are routinely screened in potential donors and are frequently knowingly transmitted because, for the most part, there are effective treatments for these viruses in immunosuppressed hosts. Thus, despite a relatively efficient transmission of these viruses and documented deaths that are directly related to them, donors testing positive for these viruses are routinely considered suitable [55].

- *Hepatitis B virus (HBV)*: Approximately 5% of people worldwide are chronically infected with hepatitis B. Overall, 15% of those chronically infected go on to develop cirrhosis, and an additional 20% will require LT. Acquisition of the HBV remains a concern after LT because the majority of the infections occur via transmission by the donor liver [56], but some donors with past exposure to HBV infection can be used selectively in some recipients.

Donors who are hepatitis B surface antigen negative (HBsAg-) but hepatitis B core antigen positive (anti-HBc+) have transmitted HBV infection to liver recipients who are HBsAg- at a rate of 33% to 78% [57]. Early studies of the use of hepatitis B core antibody positive allografts to treat HBV+ recipients suggested that the risk of HBV transmission was extremely high and carried a high mortality. However in patients who are immune to HBV (previous vaccination) it has been found to be safe to use these organs [58]. In recipients with active HBV infection or in desperate circumstances these organs have been used safely in combination with antiviral prophylaxis and immunoglobulins [55,59,60]. Additionally, donors with positive hepatitis B surface antibody (anti-HBs) do not appear to transmit HBV infection after LT [59].

The development of combined prophylaxis with hepatitis B immune globulin (HBIG) and lamivudine has proved effective not only against HBV recurrence but also against de-novo HBV infection or transmission in recipients of anti-HBcAb+ livers [59,61,62]. Nery et al., [61] reported that of 62 recipients of anti-HBc+ livers, 60 were serologically free of HBV infection under combined or lamivudine monotherapy. These data suggest that the use of HBcAb + grafts are comparable with core antibody negative grafts and that survival was improved with dual immunoprophylaxis [44,55]. In addition, Prieto et al., [56] reported that post-transplant HBV infection developed in 15 of 30 recipients of livers from anti-HBcAb+ donors compared with 3 of 181 livers from anti-HBcAb-donors. Recipients of livers from anti-HBc+ donors are at high risk for acquiring HBV infection, whereas recipients of livers from anti-HBs+ donors are significantly less likely to acquire HBV infection, and this latter group may play a role in expanding the donor pool [1].

- *Hepatitis C virus (HCV)*: About 5% of all potential organ donors are positive for antibody to HCV [63], and the transplantation because of HCV cirrhosis has increased because of the greater prevalence of the virus in the last 15 years [64]. Initially, the use of HCV+ donor organs in LT was a source of great controversy and not commonly practiced. Underlying this practice was a concern for increased risk of aggressive viral recurrence in patients receiving HCV+ grafts. LT for recipients with HCV cirrhosis from HCV+ donors were found to provide graft survival that is equivalent to HCV- grafts to HCV+ recipients [65]. Short-term studies in the early 1990s showing no difference in outcomes of HCV+ grafts and increasing donor shortages allowed for the use of HCV+ donor grafts in recipients with HCV to expand the donor pool. Long-term follow-up in the late 1990s confirmed that the use of grafts from HCV+ donors is safe and that patient and graft survival are not affected [66]. Recurrence rates of hepatitis C, manifested by mild chronic hepatitis, fibrosis, or cirrhosis have been reported to be 54.55% in HCV+ donor grafts when compared with 41.74% in HCV- grafts. Patient and graft survival at 4 years post-transplant in HCV+ donor grafts have been shown to be 83.9% and 71.9% versus 79.1% and 76.2% in HCV- donor grafts [66]. Similar rates of HCV recurrence, patient survival, and graft survival have been reported by different centers using HCV+ liver grafts for patients requiring transplantation for HCV cirrhosis [65]. Moreover, in a report by Marroquin et al., [67] showed that patient survival at 2 years was significantly higher in HCV+ recipients of HCV+ grafts than in HCV+ recipients of HCV- grafts (90% vs 77%). In contrast, other studies indicated that in patients with HCV related liver disease, there was no significant patient survival difference between the patients who received HCV+ grafts and who received HCV- grafts [68].

In general, it is obvious that livers from donors with active on going hepatitis and/or fibrosis should not be used for transplantation. In donors with a history of such infection, there have been recommendations for a routine liver biopsy before use of a graft for transplantation. A scoring system has been derived in order to aid the decision of whether a graft should be used for transplantation in this setting [69].

## Donor with Malignancies

Quantification or calculation of the true risk of donor transmitted malignancies has been difficult because of underreporting to the Organ Procurement and Transplantation Network/UNOS registry [1].

The transmission of donor-derived malignancies to recipients with catastrophic outcomes has been reported [70]. Certain tumor types, such as glioblastoma, astrocytomas and medulloblastoma, as well as tumours that have breached the blood brain barrier following ventriculoperitoneal shunts or surgery along with cerebellar tumours and previous prolonged chemotherapy for such tumours carry a higher risk of transmission and should be avoided unless the recipient status warrants the extra risk [71, 72].

According to UNOS database, 2.7% of deceased donors have a history of cancer. Between 2000 and 2005, grafts from donors with a history of malignancy were used in 891 liver transplants. The most common cancers were nonmelanoma skin cancer (n = 306) followed by central nervous system (CNS) malignancies (n = 179) and carcinoma of the uterine cervix (n = 108). Forty-five donors had a history of melanoma [73]. Presumably, none of the donors had any evidence of active malignancy, with the exception of nonmelanoma

skin cancers such as basal cell carcinoma and squamous cell carcinoma and CNS malignancy. During the study period, only two donors transmitted a fatal malignancy to recipients [73]. Given earlier reports, livers from donors with a history of melanoma or glioblastoma should not be used for transplantation [73, 74].

Buell et al., [75] have reported an overall transmission rate of CNS tumors of 23%. If donors have high-grade malignancies and/or risk factors, recipients face an increased incidence of tumor transmission of 53%. Risk factors include surgical shunts, previous craniotomy, or previous prolonged chemotherapy. These high-risk donors also should be avoided.

It is left to the judgement of the transplanting team that determine the use of these organs under certain circumstances.

## Technical Variant Grafts

In an attempt to expand the size of the donor pool, a number of surgical techniques have been developed over the past 15 years, including SLT and LDLT [76]. Couinaud's [77] anatomical classification and later refined by Bismuth [78] permits the creation of partial liver grafts from either deceased or living donors. Couinaud's classification divides the liver into eight independent segments, each of which has its own vascular inflow, outflow, and biliary drainage [77]. Segments IV to VIII are used for adults, whereas left lateral lobes (segments II and III) or left lobes (segments II, III, and IV) are used for pediatric recipients. Bleeding, bilomas, and portal vein thrombosis are complications related to the procedure itself, which are associated with an increased number of re-operation.

### Split-Liver Transplantation

Initially, Bismuth and Houssin in 1984, transplanted the left lateral segment of the left liver lobe from a cadaveric donor into a small child and discarded the remainder of the donor liver [79]. But the first SLT, was performed in 1988 allows the division of the one adult donor liver together with its vascular and biliary structure, in two functional grafts [80]. This was a new approaches for expanding the supply of liver grafts for children without affecting the supply for adults [81], but the use of one liver to obtain two grafts is limited by the fact that a small number of children and a large number of adults are candidates for LT. Therefore SLT is a well-established technique for addressing the organ shortage, but because of technical and logistic issues in both donors and recipients, accounts for only 4% of LT [82].

SLT is performed either *ex situ* or *in situ*. Both have different but distinctive advantages and disadvantages and the choice depends on surgeon preference, centre infrastructure and geographical location [9]. While splitting was originally performed as an *ex vivo* procedure, the initial results was poor because of a high incidence of PNF of the graft and some technical problems [83]. A modified *in situ* liver splitting was introduced to decrease cold ischemic time, prevent blood loss after reperfusion, reduce biliary complications and decreases the rate of PNF as compared with *ex situ* apportioning [84]. It is performed by transplanar dissection and careful preservation of lobar vasculature and biliary anatomy before aortic cross-clamp

and preservative flush. So far, there is no consensus on which technique is superior because both techniques demonstrate similar patient and graft survival rates compared with whole liver grafting [80].

Conventionally, the majority of livers have been split to produce a left lateral segment or left split grafts (segments II and III) for a child [85] and a right lobe graft (segments I and IV-VIII) for an adult recipient [86], with excellent outcomes (Figure 2). The use of SLT and live donation has greatly reduced mortality of children waiting for LT [85]. Oswari et al., [87] show that in a total of 251 liver transplants, 30 were split grafts. In the same line, Yersiz et al., [88] reported on 100 livers that were split *in situ*, yielding 190 grafts for transplantation. Left lateral segments grafts were transplanted to pediatric recipients and right lobe graft were transplanted to older children and adults. Patient and graft survivals were equal to those in 1086 recipients of cadaver whole-organ grafts during the same time period. Wilms et al., [86] compared the outcome of 70 right lobe grafts and 70 whole-liver grafts in adults. At a mean of 36 months, 2-year patient and graft survivals were similar between SLT and whole LT. There was no increased incidence of graft dysfunction secondary to small-for-size syndrome, because the right lobe grafts contained approximately 80% of the standard liver volume. Wilms et al., [86] concluded that SLT did not put adult recipients at increased risk of morbidity or mortality. Overall many studies support the view that split grafts have an equivalent outcome to whole grafts [89].

Successful SLT in two adults has also been reported [89-91]. Humar et al., [89] observed good outcomes, with no PNF, in 10 of 12 adult *in situ* split-liver recipients. Azoulay et al., [90] also reported acceptable results in 34 adult recipients of grafts split either *ex situ* (n = 30) or *in situ* (n = 4). PNF occurred in 3 of 17 left split-liver grafts, but in none of the 17 right split-liver grafts. Graft survival of left split-liver grafts at 2 years was inferior to whole-liver grafts (43% vs 85%) and was adversely affected by graft steatosis and a graft-to-recipient body weight ratio of less than 1%.

Left and right lobe splitting for two adult recipients have a high risk of small-for-size syndrome [91] because the liver is of large volume and the metabolic demands for the individual are not met. Small for size can be determined as either a graft weight/recipient weight ratio of less than 0.8% [92] or a graft volume/standard liver volume ratio of less than 40% [93]. High rates of hepatic artery thrombosis, PNF and biliary complications reflect the technical difficulties to be overcome, in addition to problems associated with small-for-size syndrome [91]. It is also noted that biliary complications are higher in split grafts compared to whole grafts with an incidence up to 26% in some series [87, 94]. One strategy to reduce this problems, is use these partial grafts for patients deemed as “less sick,” such as with lower Model for End-stage Liver Disease (MELD) scores with the thought that they are better able to tolerate the complications of SLT and the need for reoperation [89].

The acceptable outcomes make SLT as an immediate option to expand the donor pool, with high impact from both cadavers and living donors. SLT for two adults can increase the number of recipients and is possible in about 15% of optimal cadaver donors [95]. Moreover, if this technique is widely used, the supply of liver grafts from cadaveric donors could be almost doubled.

## Living-Donor Liver Transplantation

LDLT offers another source of liver grafts that can be used to expand the donor pool [24], is unique because the liver donor graft is directed to only one specified candidate, obviating the need for an allocation system. The introduction of LDLT has been one of the most remarkable steps in the field of LT. Initially described in 1969 by Smith [96] using animal models and the first human cases were reported in 1989 by Raia et al., [97]. In 1996, Lo et al., [98] performed the first successful LDLT using an extended right lobe from a living donor for an adult recipient.

The application of LDLT is associated with some well-documented advantages: 1) Transplantation can be performed on an elective basis before serious decompensation of the recipient (i.e. optimal timing; no waiting time). 2) Graft is of excellent condition (i.e. preselected organ quality and healthy donor) [14]. 3) Ischemic time is short (i.e. complications because of preservation injuries are absent). 4) Possibility to schedule surgery electively of LT for recipients. 5) Extended indications, who might otherwise not be eligible for standard deceased donor LT (i.e. reduced risk of the recipient dying on the waiting list and allowing the recipient to be medically stabilized) [99]. However, LDLT has disadvantages as well: a higher rate of surgical complications for both the donor and recipient, a higher incidence of vascular (5-15%) and biliary (10-30%) complications and a potential risk of small-for-size syndrome [100] in which the recipient fails to sustain adequate metabolic function. Small grafts require posterior regeneration to restore the liver/body ratio and it's well known that I/R significantly reduce liver regeneration. Therefore, careful selection of the donor and recipient is crucial to minimize risks and complications to obtain acceptable outcomes in LDLT [42] and favoring the recovery and functioning of the transplanted organ. The complications of adult LDLT are higher than for whole graft transplantation [9]. Some technical advances required to overcome initial problems encountered with LDLT has led to the use of techniques like middle hepatic vein inclusion or reconstruction, hemi-portocaval shunts, splenic artery ligation or dual grafts [101, 102].

Initially, the recipients in LDLT were mostly children, but with growing expertise with right lobe donation, LDLT today is also a valuable option for adult recipients, although mortality is higher to for adult to adult compared to adult to child donation. This is explained by the fact that adult to adult donation mostly encompasses a right lobe (for the graft-size disparity) and adult to children mostly a left lobe donation.

Left lobe donation has been associated with a lower mortality, compared to right lobe [100]. However, the incidence of small for-size syndrome was 25% in left-lobe recipients versus 6% in right-lobe recipients [103]. Comparing outcomes of adult-to-adult LDLT versus deceased donor LT, the graft survivals was of 87% and 81%, respectively [104]. Also, Foster et al., [105] compared the outcomes after adult-to-adult LDLT to those after deceased donor LT using nationwide databases. The patient survival rates after LDLT were similar to those after deceased donor LT. Man et al., [106] found that patients implanted with grafts less than 40% of standard liver weight suffered from transient portal hypertension after reperfusion and inflammation, which may account for the small-for-size graft injury.

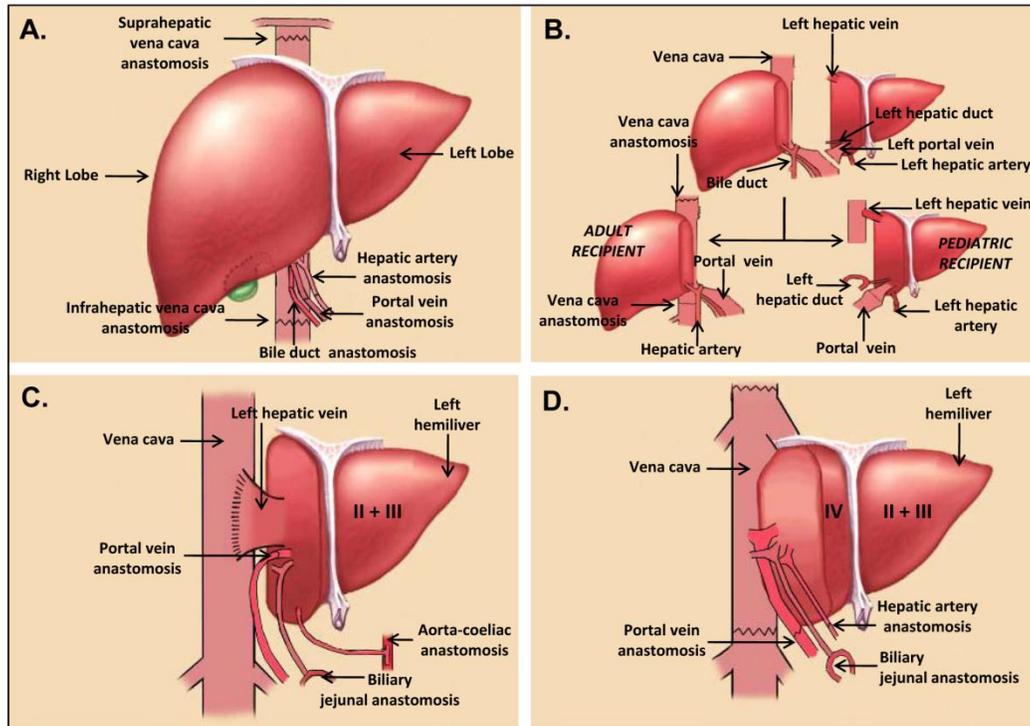


Figure 1. A) Standard technique of whole liver implantation. B) Split liver transplantation into an adult and pediatric recipient. C) Reduced-size segmental graft technique. D) Orthotopic reduced-size transplant using the left hemiliver.

## Role of Ischemia-Reperfusion Injury in Liver Transplantation from Marginal Donors

As mentioned in this review, I/R affect negatively damage and regeneration in marginal livers submitted to transplantation. A large number of factors and mediators play a part in liver I/R injury [107]. The relationships between the signaling pathways involved are highly complex and it is not yet possible to describe, with absolute certainty, the events that occur between the beginning of reperfusion and the final outcome of either poor function or a non-functional liver graft.

Cold preservation decreases metabolic activity 10-fold, and increases anaerobic metabolism and lactic acidosis, therefore resulting in mitochondrial energy uncoupling. Depletion of ATP during ischemia causes loss of transcellular electrolyte gradients, influx of free calcium and the subsequent activation of phospholipases, and therefore is the main contributor for cell swelling and lysis. Ischemia creates the basis for the subsequent production of toxic molecules after reperfusion, particularly reactive oxygen intermediates, the basis of the cascade of events that characterize the I/R injury. Even with the most effective preservation solutions, cold storage aggravates graft injury at the time of transplantation.

The prolonged times of ischemia affect negatively the post-transplant outcome from marginal livers. Indeed, liver grafts with more than 14 h of cold ischemia have been consistently associated with a two-fold increase in preservation damage resulting in prolonged postoperative course, biliary stricture, and decreased graft survival. The length of cold preservation has been associated with sinusoidal cell damage and hypercoagulability [1]. The vulnerability of individual grafts to cold ischemia time varies depending of the type of the liver. Total ischemic times of less than 12 to 16 h are well tolerated by donor livers without any risk factors, but not by marginal grafts. In liver preservation with University of Wisconsin (UW) solution, the incidence of I/R injury and PNF is quite low if recipients are transplanted with non-marginal grafts. In marginal grafts, however, with such risk factors as steatosis, donor age, donation after cardiac death donor, and reduced size, it is essential that cold ischemia time be minimized [42].

Several hypotheses have been suggested to explain the decreased tolerance of marginal liver to reperfusion injury. For instance, in the case of steatotic livers, the impairment of the microcirculation is considered a major event of reperfusion injury [108]. A reduction in hepatic microcirculation has been observed in human fatty donor livers and in experimental models of hepatic steatosis. In addition, fatty accumulation in the cytoplasm of hepatocytes as occurs in steatotic livers or elderly donors, is associated with an increase in cell volume that reduces the size of the hepatic sinusoid space by 50% compared with a normal liver and may result in partial or complete obstruction of the hepatic sinusoid space altering the infrastructure of the cell itself by displacing the surrounding organelles [108]. This cause a hepatic blood flow reduction. During transplantation, the inherently high microvascular resistance and markedly reduced flow in the sinusoidal lumen of the fatty liver might lead to impaired perfusion by cold preservation solution during organ retrieval. Blebs and solidified fat globules released into the sinusoidal space at the time of hypothermia further compromises the microvascular space and impairs hepatic microcirculation [108]. It has been postulated that marginal livers are more susceptible to lipid peroxidation because of either their lower antioxidant defenses or their greater production of reactive oxygen species (ROS) from mitochondria or xanthine/xanthine oxidase (XDH/XOD) system or both [109]. Neutrophils have been involved in the increased vulnerability of steatotic livers to I/R injury, especially in alcoholic steatotic livers. Increased endoplasmic reticulum (ER) stress may be involved in the sensitivity of other marginal grafts to I/R injury, such as steatotic livers grafts and liver grafts from aging donors. Indeed, aging donors have an increased incidence of steatosis, which may favor cold preservation injury [110]. Alterations in the activation of inflammatory transcription factors and expression of cytoprotective proteins, increased intracellular oxidants and decreased mitochondrial function and protein misfolding accumulation, and aggregation also characterize many age-related diseases [111]. Differences were also observed when we analyzed the role of the renin-angiotensin system (RAS), as the nonsteatotic grafts exhibited higher angiotensin-II (Ang-II) levels than steatotic grafts whereas steatotic grafts exhibited higher Ang-(1-7) levels [112]. In the context of I/R injury associated with LT, the axis ACE-Ang II-ATR and ACE2-Ang-(1-7)-Mas play a major role in nonsteatotic and steatotic grafts, respectively. Moreover, reduced retinol binding protein 4 (RBP4) and Toll-like receptor 4 (TLR4) levels and increased peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) levels were observed in steatotic livers compared to non-steatotic livers [113, 114].

In NHBD, the heart has stopped when aortic flush commences. During this time, metabolic activity persists in an anoxic environment leading to an increase in intracellular acidosis and accumulation of lactate. Aerobic metabolism converts to an anaerobic state, and ATP stores are rapidly depleted leading to cellular electrolyte imbalances and an increase in harmful inflammatory mediators and proteases ultimately resulting in cell death. In cold preservation at 4°C, ATP stores are depleted less rapidly [115]. All these aspects make the organs suffer severe ischemic insult. It is necessary to assess the parameters involved in the development of PNF in livers from NHBDs in order to improve their viability. The risk of PNF is unacceptably high (>50%) when livers are exposed to >30 minutes of warm ischemia before a short cold ischemia period. In a porcine NHBD LT model, the cold preservation of liver grafts is shortened from 20 to 12 to 6 h when warm ischemia time is prolonged from 10 to 20 to 30 minutes. Only liver grafts within these time limits could be safely transplanted [116]. Ma et al., [117] investigated the histological and ultrastructural characteristics of liver grafts during different warm ischemia times in rats and found that the morphological changes are positively related to warm ischemia injury in a time-dependent manner during the reperfusion period. Therefore they consider that a rat liver graft undergoing warm ischemia injury is in the reversible stage when the warm ischemia time is within 30 minutes. A 45-minute warm ischemia time may be a critical point for a rat liver graft to endure warm ischemia injury. When the warm ischemia time is over 60 minutes, the damage is irreversible. In NHBDs, PNF is associated with more activated Kupffer cells in recipients, by higher production of tumor necrosis factor (TNF- $\alpha$ ) and interleukin-6 (IL-6), with lower alpha-tocopherol and reduced glutathione [116].

## **Surgical and Pharmacological Strategies in Liver Transplantation from Marginal Donors**

Effective measures have been taken to improve outcomes when using these marginal livers, include rapid cooling after arrest and minimization of both cold (<8 h) and warm ischemia time. In addition several approaches are being explored to improve the viability of marginal liver grafts, including improvements in donor organ perfusion and preservation methods, additives to preservation solution, pharmacological treatments (modulators of rennin-angiotensin system, modulators of activating pro-survival kinase cascades, adipocytokines derived from liver or adipose tissue, antiapoptotic strategies, inflammatory cytokines, energy status enhancement, microcirculation amelioration, antioxidant usage), gene therapy, surgical technicals (i.e. ischemic preconditioning) and others.

### **Static Organ Preservation and Preservation Solutions**

Static cold storage (SCS) is the most commonly used preservation method used for all organs. The principles underlying cold preservation are the slowing of metabolism (by cooling) and the reduction of cell swelling due to the composition of preservation solutions. The introduction of the UW solution by Belzer for SCS was a breakthrough and remains the

conventional method of preservation [118]. Some additives used in preservation solutions for marginal liver grafts are listed in table 2 and show below.

- *Trimetazidine and AICAR*: Trimetazidine (TMZ), has been used as an additive in UW solution to protect steatotic livers exposed to prolonged cold ischemia in an *ex vivo* model of hepatic ischemia [119]. This could be of interest since irreversible injury has been reported in liver grafts preserved in UW after prolonged cold ischemic periods (between 16 h to 24 h) [119]. Studies examining the underlying protective mechanisms of TMZ suggest that mitochondria, energy metabolism, oxidative stress and microcirculation might be important targets through which TMZ exerts its cytoprotective effect [119]. Similarly to the benefits of TMZ, the addition of AMP-activated protein kinase (AMPK) activators to UW solutions such as 5-amino-4-imidazole carboxamide riboside (AICAR), protected steatotic livers against their vulnerability to I/R. TMZ, by means of AMPK, increased nitric oxide (NO), thus protecting steatotic livers against their vulnerability to I/R injury [119]. Taking these observations into account, TMZ and AICAR may constitute new additives to UW solution in steatotic liver preservation, whereas a combination of both seems unnecessary.

- *Polysol and Glucagon*: Hata et al., [120] show that Polysol preservation substantially suppressed the deleterious mitochondrial alterations in steatotic livers resulting in significantly better integrity and function. Also glucagon has been used as additive in UW solution to increase the cAMP signal in the liver. Upon reperfusion, liver integrity significantly improves after glucagon administration, with 66% reduction in transaminases and a threefold increase in hepatic bile production as compared with untreated livers. Treatment of damaged livers by glucagon enhances cAMP tissue levels during ischemic preservation and improves hepatic integrity upon reperfusion. This may represent a promising approach for the use of livers from NHBDs in clinical transplantation [121].

- *Serine protease and Streptokinase*: Pretreatment with serine protease inhibitors has been shown to minimize the damage caused by warm ischemia in experimental models in NHBDs [122]. Addition of antithrombotic drugs (Streptokinase) to the perfusion solutions improved the microcirculation of livers after warm ischemia and may thus represent a promising approach to attenuate parenchymal cell injury in liver graft retrieval from NHBDs [123]. It is well known that the integrity of liver grafts from NHBDs is additionally affected by microvascular alterations, including erythrocyte aggregation and thrombus formation, which might hamper appropriate equilibration of the preservation of grafts microvasculature, precluding cold preservation. In the same line, the elimination of Kupffer cells reduced thromboxane B2 and cytokines and improved sinusoidal microcirculation in NHBDs [124].

- *N-acetylhistidine*: Recently, a modified histidine-tryptophan-ketoglutarate (HTK) solution that contains N-acetylhistidine, amino acids and iron chelators (HTK-N) has been developed. Liu et al., [125] demonstrates that HTK-N protect liver grafts with microvesicular steatosis caused by toxic injury from cold ischemia injury better than standard HTK most likely via inhibition of hypoxic injury and oxidative stress and amelioration of the inflammatory reaction occurring upon reperfusion.

*Epidermal growth factor (EGF) and Insulin growth actor (IGF-I)*: The results, based on isolated perfused liver, indicated that the addition of EGF and IGF-I, separately or in combination to UW reduced hepatic injury and improved function in steatotic and non-steatotic types. EGF increased IGF-I, and both additives up-regulated AKT in both liver types. This was associated with glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) inhibition in non-steatotic livers and PPAR $\gamma$  over-expression in steatotic livers [126].

**Table 2. Some additives in preservation solution used as strategies in marginal donors**

Marginal Donor	Additive	Model	Effects
Steatosis	Erythropoietin ( <i>EPO</i> )	Mice Isolated Perfused	↓ Hepatic injury
	TMZ	Rat Isolated Perfused	↓ Hepatic injury, oxidative stress ↑ Cytoprotection
	AICAR ( <i>AMPK activator</i> )		↓ Hepatic injury ↑ Hepatic functionality
	TMZ and AICAR		↓ Hepatic injury ↑ Hepatic functionality
	EGF		↓ Hepatic injury ↑ IGF-1 and PPAR $\gamma$
	EGF and IGF-1		↓ Hepatic injury ↑ AKT
	IGF-1		↓ hepatic injury, mitochondrial damage, oxidative stress
	Polysol		↓ Hepatic injury, integrity ↑ Hepatic functionality, cAMP
	N-acetylhistidine		Rat LT
	Tauroursodeoxycholate acid ( <i>Bile acid</i> )	↓ Hepatic injury, endoplasmic reticulum stress	
NHBD	Glucagon	Rat Isolated Perfused	↓ Hepatic injury ↑ Hepatic functionality, cAMP
	Streptokinase ( <i>antithrombolytic drug</i> )		↓ Hepatic and parenchymal injury ↑ Microvasculature
	Nafamostat mesilate ( <i>Serine protease inhibitor</i> )		↓ Induction inflammatory cytokines ↑ Sinusoidal microcirculation
	FR167653 ( <i>Protein kinase inhibitor</i> )		↓ Induction cytokines ↑ Sinusoidal microcirculation
	Meloxicam ( <i>COX-2 inhibitor</i> )		↓ Hepatic injury, oxidative stress, apoptosis
	CGS 21680 (A2 receptor agonist)	Pig LT	↓ Hepatic injury ↑ Bile production
	OP-2507 ( <i>Prostacyclin analogue</i> )		↓ Hepatic injury ↑ Survival, hepatic microcirculation

## Pharmacological Treatments

- *Modulators of Renin-Angiotensin System:* Previous researches have observed an important role for the RAS, known for its regulation of blood pressure and fluid homeostasis, in both I/R injury and liver regeneration after partial hepatectomy [127]. In conditions of partial hepatectomy under I/R, Angiotensin receptors (AT1R and AT2R) antagonists for steatotic livers improved regeneration in the remnant liver. AT1R antagonist, through NO inhibition, protected steatotic livers against oxidative stress and damage. The combination of AT1R and AT2R antagonists in steatotic livers showed stronger liver regeneration than either antagonist used separately and also provided the same protection against damage as that afforded by AT1R antagonist alone. These results could be of clinical interest in liver surgery [127]. BK seems to be a key mediator in the benefits of all the blockers of Ang-II activity (ACE inhibitors, AT1R antagonists, and AT2R antagonists) in steatotic livers undergoing I/R [128]. In LT, Ang-II is an appropriate therapeutic target only in non-steatotic livers. It was observed an upregulation of ACE2 in steatotic liver grafts, which was associated with decreased Ang-II and high Ang-(1-7) levels. Ang-(1-7) receptor antagonist reduced necrotic

cell death and increased survival mediated by NO inhibition in recipients transplanted with steatotic liver grafts. These results indicate a novel target for therapeutic interventions in LT within the RAS cascade, based on Ang-(1-7), which could be specific for this type of liver [112].

- *Adipocytokines derived from liver and/or adipose tissue:* To date, adipose tissue has been considered the major site for endogenous adiponectin production, although there are other potential sources, including the liver [129]. A recent study indicated that steatotic livers can generate adiponectin as a consequence of I/R [129]. The role of adiponectin in hepatic I/R injury remains unclear. PPAR $\alpha$  agonists, through PPAR $\alpha$ , inhibited mitogen (MAPK) expression following I/R. This in turn inhibited the accumulation of adiponectin in steatotic livers and reduced its negative effects on oxidative stress and hepatic injury [129]. However, another study by Man et al., [130] in small fatty grafts, adiponectin treatment exerted anti-inflammatory effects that down-regulated TNF- $\alpha$  mRNA and vasoregulatory effects that improved the microcirculation. Adiponectin anti-inflammatory effects also include the activation of cell survival signaling via the phosphorylation of Akt and the stimulation of NO production. Thus, on the basis of the different results reported to date in hepatic I/R, it is difficult to discern whether we should aim to inhibit adiponectin, or administer adiponectin to protect steatotic livers against cold ischemia associated with LT (Table 3).

RBP4 is an adipokine synthesized by the liver, whose known function is to transport retinol in circulation. However, the role of RBP4 in the liver is largely unknown. A recent study indicated that steatotic liver grafts were found to be more vulnerable to the down-regulation of RBP4 and the over-expression of PPAR $\gamma$ . RBP4 treatment (through AMPK induction) reduced PPAR $\gamma$  over-expression, thus protecting steatotic liver grafts against I/R injury associated with LT. In terms of clinical application, therapies based on RBP4 treatment and PPAR $\gamma$  antagonists might open new avenues for steatotic LT and improve the initial conditions of donor livers with low steatosis that are available for transplantation [113].

- *Antiapoptotic strategies:* Recent studies indicated that 4-phenyl butyric acid (PBA), and especially tauroursodeoxycholic acid (TUDCA), reduced inflammation, apoptosis and necrosis, and improved liver regeneration in both steatotic and non-steatotic livers in partial hepatectomy under vascular occlusion. Both compounds, especially TUDCA, protected both liver types against ER damage, as they reduced the activation of two of the three pathways of UPR (namely inositol-requiring enzyme and PKR-like ER kinase) and their target molecules caspase 12, c-Jun N-terminal kinase and C/EBP homologous protein-10. Only TUDCA, possibly mediated by extracellular signal-regulated kinase upregulation, inactivated glycogen synthase kinase-3 $\alpha$ . This in turn, inactivated mitochondrial voltage-dependent anion channel, reduced cytochrome C release from the mitochondria and caspase 9 activation and protected both liver types against mitochondrial damage [110]. Also, strategies aimed at modulating component of ER stress-mediated cell death could protect not only against ER stress but also against the mitochondrial-dependent apoptosis pathway. Further studies will be required to elucidate whether these chemical chaperones such as PBA and TUDCA could be considered as useful strategies in clinical LT. They have been used for clinical treatment of urea cycle disorders, cholestatic liver diseases and cirrhosis [110]. Results of clinical trials have shown that PBA has few side effects and is safe for patients since it is well tolerated at high dose for long periods of time [131]. TUDCA is a derivate of an endogenous bile acid, and it has been safely used as a hepatoprotective agent in humans with cholestatic liver diseases [132]. On the other hand, ER stress could be not involved in the protective mechanisms of TUDCA in

steatotic LT with liver grafts subjected to 6 h of cold ischemia in UW solution. Indeed, a recent study by Jimenez-Castro et al., [114] shown that TUDCA only protected steatotic livers grafts and did so through a mechanism independent of ER stress. TUDCA, which inhibited PPAR $\gamma$ , up-regulated TLR4, specifically the TIR domain-containing adaptor inducing IFN $\beta$  (TRIF) pathway. TLR4 pathway, thus protecting steatotic liver grafts. TLR4 activating-based strategies could reduce the inherent risk of steatotic liver failure after LT. This contrast with the studies from Anderson et al., [133] reported in steatotic liver grafts submitted to 2 h of cold ischemia in HTK solution, with had increased ER stress responses and markers of hepatocellular injury after LT. ER stress response components were reduced by TUDCA and this resulted in an improvement in the allograft injury. TUDCA treatment decreased NF $\kappa$ B activation and the proinflammatory cytokines IL-6 and IL-1 $\beta$  and CHOP expression (Table 3).

- *Modulation of inflammatory cytokines and oxidative stress*: Livers of mice having a spontaneous mutation in the leptin gene (ob/ob), resulting in global obesity and liver steatosis, are endotoxin sensitive, and do not survive I/R injury. It was reported that 14%-31% survival of isotypematched control mAb-treated ob/ob mice survived after 15 minutes of ischemia and 24 h of reperfusion. In contrast, 75%-83% of ob/ob mice pre-treated with an anti-LPS mAb prior to initiation of I/R survived after ischemia and 24 h of reperfusion. Furthermore, there was a decrease in ALT and circulating endotoxin levels when treated with an anti-LPS mAb compared with control antibodies. Attenuation of the endotoxin load with anti-LPS mAb, before initiation of I/R is cytoprotective and improves survival [134]. In addition, FR167653, a newly synthesized cytokinesuppressive anti-inflammatory agent, attenuates graft injury in LT from NHBDs which often involves hepatic warm I/R injury triggered by inflammatory cytokines. In porcine LT from NHBDs, microcirculatory disturbance was attenuated, liver injury was lessened, and ATP resynthesis was enhanced by the use of FR167653. In addition, FR167653 inhibited neutrophil infiltration in the liver tissue, and suppressed release of inflammatory cytokines after LT from NHBDs. The inhibitory effect of FR167653 on the release of inflammatory cytokines plays an important role in liver graft protection [135]. Ye et al., [136] explored the protective effect of high-dose reduced glutathione (GSH) and venous systemic oxygen persufflation on rat steatotic liver grafts following transplantation and effectively protect from ischemic damage and significantly improve early survival rate. In another study, Kim et al., [137] reported the cytoprotective role of heme oxygenase-1 (HO-1) induction by cobalt protoporphyrin (CoPP), an HO-1 inducer in alcoholic steatotic livers exposed to cold I/R.

- *Modulation of Kupffer cells and leukocytes* : Elimination of Kupffer cells and administration of a protease inhibitor improve graft viability and prevent reperfusion injury in NHBD. Frankenberg et al., [138] found that depletion of Kupffer cells with gadolinium chloride (GdCl $_3$ ) in donor animals prevents PNF of fatty livers after transplantation, and diminishes amino acid release at harvest, but blocks the increased expression of the adhesion molecule ICAM-1 only after transplantation. Moore et al., [139] documented that the interactions between fibronectin, a key extracellular matrix protein, and its integrin receptor  $\alpha$ 4 $\beta$ 1, expressed on leukocytes, specifically up-regulated the expression and activation of metalloproteinase-9 in a well-established steatotic rat liver model of ex vivo ice-cold ischemia followed by LT. The presence of the active form of MMP-9 was accompanied by massive intra-graft leukocyte infiltration, high levels of proinflammatory cytokines, such as interleukin-1 $\beta$  and TNF- $\alpha$ , and impaired liver function. Interestingly, MMP-9 activity in

steatotic liver grafts was to some extent independent of the expression of its natural inhibitor, the tissue inhibitor of MMP-1. Moreover, the blockade of fibronectin- $\alpha4\beta1$  integrin interactions inhibited the expression/activation of MMP-9 in steatotic LT without significantly affecting the expression of metalloproteinase-2 (MMP-2, gelatinase A). These findings reveal a novel aspect of the function of fibronectin- $\alpha4\beta1$ -integrin interactions, which is of significance in the successful use of marginal steatotic livers in transplantation [139]. Amersi et al., [140] showed the effects of connecting segment-1 (CS1) peptide in a steatotic rat model of *ex vivo* cold ischemia followed by iso-transplantation. CS1 peptide therapy significantly inhibit the recruitment of T lymphocytes, neutrophil activation/infiltration, and repressed the expression of proinflammatory TNF- $\alpha$  and IFN- $\gamma$ . Moreover, it resulted in selective inhibition of inducible nitric oxide synthase expression, peroxynitrate formation and hepatic necrosis. Importantly, CS1 peptide therapy improved function/histological preservation of steatotic liver grafts and extended survival. Also Moore et al., [141] reported that CS1 peptide in steatotic rat LT showed a profound decrease in T-cell and monocyte/macrophage infiltration and significantly reduced levels of cytokine expression such as IL-2 and IFN- $\gamma$ . Fondevila et al., [142] reported the effect of a cyclic RGD peptide with high affinity for the  $\alpha5\beta1$ , the fibronectin integrin receptor in a rat model of steatotic liver cold ischemia followed by LT. The RGD peptide therapy ameliorated steatotic I/R injury and improved the recipient survival rate. It significantly inhibited the recruitment of monocyte/macrophages and neutrophils and depressed the expression of pro-inflammatory mediators, such as inducible nitric oxide sintase (iNOS) and IFN- $\gamma$ . Xu et al., [143] reported that C3aP, a complement component stimulates hepatocyte proliferation and reversed fatty degradation of hepatocytes, thus enhancing hepatic function and prolonging the survival of recipients of steatotic LT rat (Table 3).

In a series of *ex-vivo* liver perfusions of >50% steatotic liver grafts and in a group of LT between steatotic Zucker and lean Zucker rats, the blockade of selectins by P-selectin glycoprotein ligand-1 (PSGL1-Ig) significantly improved liver function over control animals. *Ex-vivo* livers perfused with PSGL1-Ig had lower transaminase release, increased portal venous flow, and increased bile production compared with controls. Histologic architecture of the liver after 2 h of perfusion showed minimal changes in the PSGL1-Ig-treated grafts versus severe centrilobular disruption, drop-out, and necrosis in controls. Survival of lean rats who underwent transplantation with steatotic livers in the control group was only 40% compared with 90% in the same combination treated with PSGL1-Ig at harvest and before reperfusion [144].

- *Regulators of lipid metabolism*: Manipulation of the chemical composition of hepatic lipids may evolve as a useful strategy to expand the donor pool and improve the outcome after LT. Macrosteatotic livers disclosed an abnormal omega-6: omega-3 PUFA ratio that correlates with a microcirculatory defect that enhanced reperfusion injury [145]. Therefore, normalization of the  $\Omega$ -6: $\Omega$ -3 FA ratio appears to be crucial for protection of the steatotic liver from reperfusion injury. Preoperative dietary omega-3 PUFAs protect macrosteatotic livers against reperfusion injury and might represent a valuable method to expand the live liver donor pool [145]. Clavien et al., treated three live liver donors with moderate degrees of steatosis by oral administration of X-3 FAs. All donors showed a significant reduction of hepatic fatty infiltration within one month. Subsequently, LT was carried out for three candidates with uneventful outcomes for both donors and recipients. A very promising option to prevent post-transplant complications appears to be the use of a pretreatment with X-3

FAs. However, the approach is only feasible in living donation since requires oral administration of X-3 FAs before organ procurement [146]. Cerulenin has been shown by Chavin et al., [16] to reduce body weight and hepatic steatosis in murine model of obesity by inhibiting fatty acid synthase. Indeed when administered prior to I/R is adequate for protecting steatotic livers subjected to LT. Cerulenin inhibited fatty acid metabolism by down-regulating PPAR $\alpha$ , as well as mitochondrial uncoupling protein 2 (UCP2), with a concomitant increase in ATP.

- *Amelioration of microcirculation*: Fukunaga et al., [147] evaluated the effects of the endothelin antagonist TAK-044 and the platelet activating factor antagonist E5880 on the function of grafts from NHBDs in porcine LT and found that the 7-day survival rate of the recipients in treated groups was 100%. The increases of the serum concentrations of AST, lactate dehydrogenase and arterial lactate 1-4 h after LT were significantly inhibited in the treated groups. When donor livers were pretreated with a prostacyclin analogue (OP-2507) immediately before the induction of cardiac arrest and the grafts were preserved in Euro-Collins solution containing OP-2507, the survival rates after LT improved significantly [148].

- *Modulators of adenosine*: In a model of LT from NHBD pigs, Net et al., [149] evaluated the involvement of adenosine and adenosine receptors) during normothermic recirculation (NR). Application of NR after 20 minutes of warm ischemia reversed the lethal injury associated with transplantation of NHBD livers, achieving 5-day survival and diminishing glutathione S-transferase (GST), AST and hyaluronic acid. Adenosine administration prior to warm ischemia simulated the effect of NR. During NR, hepatic adenosine levels increased and xanthine levels decreased [149]. Addition of a selective A<sub>2</sub>-receptor agonist (CGS 21680) to the preservation solution reduced the biochemical parameters of hepatic damage and promoted an increase in hepatic bile production. This effect, which may represent a promising approach for the use of NHBD grafts, seems to be mediated through activation of protein kinase A [150]. In the same line, pioglitazone activated protein kinase A (PKA), increasing Multidrug resistance protein 2 (Mrp2) transport to detoxify xenobiotics and improving in macrovesicular fatty livers perfusion [151]. Arai et al., [152] show the adenosine A<sub>2</sub> receptor agonist (CGS-21680) and dibutyryl-cyclic adenosine monophosphate (DB-cAMP) decreased sinusoidal endothelial cell killing to the same after cold liver storage in a steatotic rat model (Table 3).

- *Improvement of energy status*: S-adenosyl-L-methionine (SAME) administration had beneficial effects on I/R injury associated with pig LT from NHBDs. SAME reduced hepatic damage, apoptosis of hepatocytes and sinusoidal endothelial cells after reperfusion. SAME increased energy charge at the end of NR and favored the balance between adenosine and xanthine. It was also associated with higher portal blood flow and improvements on biliary tract damage [153]. AICAR, increased AMPK and constitutive NOS activities and protected against lipid peroxidation, nitrotyrosine formation and hepatic injury in steatotic LT. This indicates that AICAR as a pharmacology strategy in steatotic LT [154]. In contrast with previous studies in normothermic hepatic conditions, the benefits of AICAR were not related with changes in ATP. In a recent study from our group in steatotic liver TR we have shown that the blockage of cAMP generation by adenylate cyclase inhibitor pre-treatment had the following results: reduced hepatic injury and increased survival of steatotic graft recipients; greater preservation of ATP and reduced lactate accumulation through cold ischemia. This blockage of cAMP by a nitric oxide-dependent mechanism protected steatotic liver grafts against oxidative stress and microvascular disorders after reperfusion [155].

**Table 3. Some pharmacological and gene therapy strategies used in marginal liver grafts**

Marginal Donor	Strategy	Model	Effects
Steatosis	Adenosine A2 receptor agonist	Rat	↓ Sinusoidal endothelial cell killing
	CGS-21680	Isolated	↓ Sinusoidal endothelial cell killing
	BD-cAMP	Perfused	↓ Sinusoidal endothelial cell killing
	Pioglitazone		↓ Hepatic injury ↑ PKA and Mrp2
	CoPP ( <i>HO-1 inducer</i> )		↓ Hepatic injury and oxidative stress
	PSGL1-Ig		↓ Hepatic injury ↑ Survival, liver function
	Cerulein	Mice LT	↓ Hepatic injury, PPAR $\alpha$ , UCP2 ↑ Survival, ATP
	TUDCA	Rat LT	↓ Hepatic injury, ER stress, inflammation, NF $\kappa$ B, IL-6, IL-1 $\beta$ ,
	TLR4 agonist		↓ Hepatic injury
	C3aP		↓ Fatty degradation ↑ Survival, hepatocyte proliferation
	Adenylate cyclase inhibitor		↓ Hepatic injury, oxidative stress, lactate accumulation, microvascular disorders ↑ Survival, preserve ATP
	Adiponectin		↓ Liver injury, inflammation ↑ Microcirculation, NO production
	FTY720		↓ Acute phase inflammatory cascade ↑ Akt
	RBP4		↓ Liver injury, PPAR $\gamma$
	Ang-(1-7) receptor antagonist		↓ Oxidative stress, necrotic cell death ↑ Survival
	AICAR		↓ Hepatic injury, oxidative stress ↑ AMPK, constitutive NOS
	CS1		↓ Oxidative stress, necrosis, inflammation, TNF- $\alpha$ , IFN- $\gamma$ ↑ Survival
	CS1		↓ T-cell, monocyte/macrophage infiltration, IL-2 and IFN- $\gamma$
	RGD peptide		↓ Hepatic injury, neutrophils, recruitment of macrophages ↑ Survival
	GSH		↓ Hepatic injury ↑ Survival
GdCl3	↓ Liver injury, ICAM-1, amino acid release at harvest		
Fibronectin	↓ Hepatic injury ↑ Activation and expression of MMP-9		
HO-1 (adenoviral transfer)	↓ Hepatic injury, apoptosis, macrophage infiltration, iNOS, caspase-3, oxidative stress ↑ Survival, Bcl-2 and Bag-1		
SOD1 (adenoviral transfer)	↓ Necrosis, apoptosis, NF $\kappa$ B, JNK, ROS ↑ Survival		
Elderly	Melatonin	Rat LT	↓ Oxidative stress, inflammatory response
	HO-1		↓ Hepatic injury, apoptosis, inflammation, oxidative stress
NHBD	Pentoxifylline	Rat LT	↓ Hepatic injury ↑ Survival
	L-arginine	Pig LT	↓ Hepatic injury and Biliary tract damage
	TAK-044 ( <i>Endothelin antagonist</i> )		↓ Hepatic injury, lactate dehydrogenase ↑ Survival
	E5880 ( <i>PAF antagonist</i> )		↓ Hepatic injury, lactate dehydrogenase ↑ Survival
	OP-2507 ( <i>Prostacyclin analogue</i> )		↓ Hepatic injury ↑ Survival, hepatic microcirculation
	FR167653 ( <i>Protein kinase inhibitor</i> )		↓ Liver injury, microcirculatory damage, neutrophil infiltration, inflammation
	Adenosine		↓ Hepatic injury, GST, AST, hyaluronic acid
	SAME		↓ Hepatic damage, apoptosis ↑ Energy charge, portal blood flow

## Gene Therapy

Advances in molecular biology provide new opportunities to reduce liver I/R injury in marginal donors using gene therapy.

- *Antioxidant therapy (SOD, HO-1)*: Genetic modification of fatty livers using viral vectors is a new approach to protecting marginal grafts against PNF. Endogenous radical scavengers such as superoxide dismutase (SOD) degrade ROS; however, SOD is destroyed rapidly when given exogenously. Therefore, an adenoviral vector encoding the Cu/Zn-SOD gene (Ad.SOD1) was used in a rat model of fatty LT. Ad.SOD1 treatment increased survival dramatically, blunted transaminase release, and reduced necrosis and apoptosis significantly. Free radical adducts increased 2-fold in the ethanol group compared with untreated controls. Ad. SOD1 blunted this increase and reduced the activation of NF- $\kappa$ B. However, release of TNF- $\alpha$  was not affected. Ad.SOD1 also blunted JNK activity after LT. Gene therapy with Ad.SOD1 protects marginal livers from failure after transplantation because of decreased oxygen radical production [156]. Lehmann et al., [157] identified the isoform with the highest effectiveness against I/R injury after fatty LT. Some donors were infected with adenoviruses expressing either the gene lacZ encoding bacterial beta-galactosidase (Ad.lacZ), Ad.SOD1, Ad.SOD2 (mitochondrial isoform), or Ad.SOD3 (extracellular isoform). Ad.SOD1 treatment increased survival and reduced necrosis, whereas Ad.SOD3 had no protective effect. Ad.SOD2 was not as protective as Ad.SOD1. Hence cytosolic SOD represents the most effective isoform of SOD to protect transplanted livers from failure; this may be related to lowered NF- $\kappa$ B and JNK activity because of reduced oxygen-derived radical production.

HO-1, as a cytoprotective protein may be important in ameliorating hepatic I/R injury. Ad.HO-1 gene transfer was used to analyze the effects of HO-1 overexpression in the well-established fatty Zucker rat model of I/R followed by LT. Ad.HO-1 gene therapy exhibited less macrophage infiltration in the portal areas and increased recipient survival in steatotic LT. The Ad.HO-1 group and increased the expression of antiapoptotic Bcl-2 and Bag-1 [158] thus modulating pro and anti-apoptotic pathways (Table 3).

- *Adipocytokine*: Massip-Salcedo et al., [129] demonstrated though the systemic delivery of adiponectin in livers treated with adiponectin siRNA that steatotic livers by themselves can generate adiponectin as a consequence of I/R. This study reports evidence of the injurious effects of adiponectin in steatotic livers under warm ischemic conditions, and results suggest the clinical potential of gene therapy for I/R damage in steatotic livers by siRNA-mediated adiponectin gene silencing [129]. These results could be of clinical interest. Viral vectors are associated with severe side effects. Although non-viral vectors (such as naked DNA and liposomes) are likely to present fewer toxic or immunological problems, they suffer from inefficient gene transfer [159].

## Machine Perfusion

Machine liver perfusion has emerged with promising data over the past decade as an alternative preservation method to SCS which can be further categorized based on the temperature employed and has emerged with promising data over the past decade because it has significant potential in graft preservation and optimization when the use of marginal organs is the objective. Machine perfusion involves pulsatile perfusion of the liver using a

machine as opposed to SCS. This can be performed by perfusing the liver with a hypothermic perfusate or with a normothermic perfusate. The safety and efficacy of machine perfusion compared to SCS to decrease liver I/R injury is yet to be assessed in humans by randomized controlled trials [160].

- *Normothermic Machine Perfusion (NMP)*: The first successful human LT carried out by Starzl et al., [161], were transplanted after liver graft pretreatment by machine perfusion with diluted, hyperbaric oxygenated blood. Most perfusion circuits were assembled from standard cardiopulmonary bypass components. Principle constituents are a centrifugal pump, a membrane oxygenator and a heat exchanger. Other critical components of the perfusate include nutrition (glucose, insulin, aminoacids), drugs to prevent thrombosis or microcirculatory failure (heparin, prostacyclin) and agents to reduce cellular oedema, cholestasis and free radical injury [162]. NMP maintains and mimics normal *in vivo* liver conditions and function during the entire period of preservation, thus avoiding hypothermia and hypoxia and minimizing preservation injury [160]. In contrast to SCS preservation the concept of normothermic preservation is to maintain cellular metabolism. The underlying principle is the combination of continuous circulation of metabolic substrates for ATP regeneration and removal of waste products. Imber et al., [163] found that, after 1 h of warm ischemia, porcine livers that were normothermically perfused had greater bile and factor V production, glucose metabolism, and galactose clearance than SCS. However, Reddy et al., [164] observed no difference in hepatic synthetic function among porcine livers receiving NMP before SCS compared with porcine livers preserved by SCS alone. Schön et al., [165] studied NMP to preserve pig livers for transplantation and to rescue them from warm ischemia in a model of donor after cardiac death. Short (5 h) or prolonged (20 h) NMP preservation is superior to SCS for normal and ischemically damaged livers, respectively [160]. The longest preservation of steatotic livers was the NMP preservation for 48 h in a pig model by Jamisson et al., who employed blood containing additional insulin and vasodilators as perfusate, and observed a mild reduction of steatosis from 28% to 15%. The NMP circuit dually perfuses 1.5 L of autologous heparinized blood at physiological pressures, which allows hepatic blood flow autoregulation. Prostacyclin, taurocholic acid, and essential amino acids are infused continuously. The concept of normothermic recirculation in the context of NHBDs was first developed by Garcia-Valdecasas et al., [166]. With 4 h of NMP, hepatic damage incurred during 90 minutes of cardiac arrest can be reverted, achieving 100% graft survival after 5 days of postransplant follow-up. Administration of pentoxifylline or arginine to rat and pig respectively may also improve the quality of NHBD by reversing ischemic damage [167].

- *Hypothermic Machine Perfusion (HMP)*: The first and most prominent difference between SCS and (oxygenated) HMP is the restoration of the tissue's energy charge and glycogen content while preventing ATP depletion [160]. Lee et al., [168] reported that HMP for 5 h improves survival and reduces cellular damage of liver tissue that has experienced 30 minutes of warm ischemia in NHBD rat livers. Dutkowski et al., [169] show that 45 minutes of warm in situ ischemia followed by 5 h cold storage followed by 1 h HMP improved NHBD rat livers significantly, with a reduction of necrosis, less AST release. This was associated with increased bile flow, ATP preservation and a significant extension of graft survival. Bessems et al., [170] employed HMP preservation with UW-gluconate solution or polysol on NHBD rat livers for 24 h. Both alleviated I/R and hepatocellular damage compared to SCS in UW. However, polysol was associated with less cellular edema after preservation and higher

flow during reperfusion, bile production and ammonia clearance than UW-gluconate. Extracorporeal membrane oxygenation (ECMO) may be used to reduce warm ischemia time in liver graft obtained from uncontrolled NHBDs, thereby increasing graft salvage rates. Rojas et al., [171] evaluated the use of warm blood veno-arterial ECMO reperfusion in preheparinized NHBD swine. ECMO was started after 30 or 60 minutes of cardiac arrest and kept running for 120 minutes. In this model, ECMO support restored liver perfusion, oxygenation, and bile production after 1 h of cardiac arrest. Wang et al., [172] reported a liver graft donor who was maintained on ECMO after successful cardiopulmonary resuscitation. The liver was procured using a rapid flush technique 4 h after instituting ECMO. Graft function recovered fully after transplantation. There is a substantial body of research, predominantly in rodents, demonstrating improved preservation by providing oxygen to livers [173]. Nevertheless, clear guidelines towards target values/ranges for oxygen levels regarding the optimal duration of oxygenation during HMP are lacking. HMP can also be applied at the end of the cold storage period, which is attractive for logistical reasons. The disadvantage here is the time-dependent increase in vascular resistance, bearing the risk of damage to the sinusoidal endothelium.

- *Subnormotermic Machine Perfusion (SNMP)*: SNMP preservation lies between HMP and NMP, but it remained relatively unexplored until recently despite holding promising applications [174]. In an isolated rat liver perfusion model, SNMP enhanced the functional integrity of steatotic livers compared with SCS findings. Organ protecting properties mediated by decreasing the temperature to a 20–28°C have been observed previously. SNMP avoids some of the downsides of hypothermia while maintaining mitochondrial function and it may circumvent the logistical restraints of NMP [160]. Vairetti et al., [174] preserved steatotic rat livers by SNMP (20°C) with Krebs-Henseleit solution for 6 h and obtained reduced I/R damage compared to SCS.

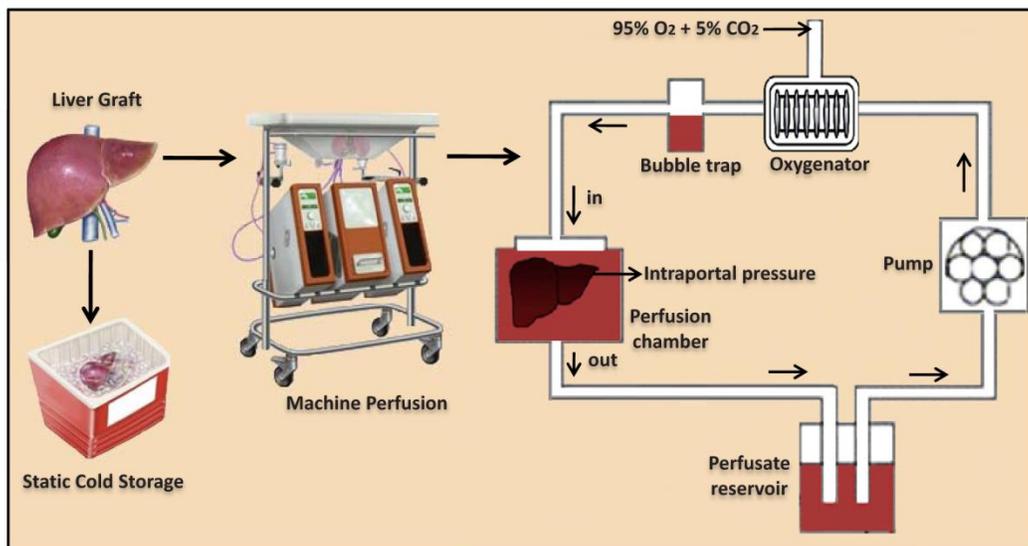


Figure 2. Illustrative representation of machine reperfusion and static cold storage.

## Surgical Strategies

The induction of consecutive periods of ischemia to the liver does not provoke an additive effect in terms of the hepatocyte lesion. Ischemic PC based on a brief period of ischemia followed by a short interval of reperfusion prior to a prolonged ischemic stress protects against hepatic I/R injury [3]. The molecular basis for PC consists of a sequence of events: in response to the triggers of PC, a signal must be rapidly generated which is then transduced into an intracellular message leading to the amplification of the effector mechanism of protection [175, 176]. As in the pathophysiology of hepatic I/R, in the modulation of hepatic injury induced by IP there is a complex interaction between different cell types. Vasoactive substances such as adenosine, NO, bradykinin, etc, have been considered the major players in triggering preconditioning [176]. In addition to the extracellular mediators, PC involves activation of intracellular messengers such as PKC, AMPK, p38 MAPK, Ik kinase; signal transducer and activator of transcription-3 (STAT3) and transcription factors including NFκB and heat shock transcription factor 1 (HSF1) [177]. The downstream consequences of these pathways could be cytoprotective by abrogation of cell death pathways, stimulating antioxidant and other cellular protective mechanisms including MnSOD and heat shock proteins (HSPs), and by initiating entry into the cell cycle [176]. The benefits of PC on energy metabolism, inflammatory mediators including ROS and TNF, mitochondrial dysfunction, KC activation, and microcirculatory disorders associated with I/R injury have also been described in steatotic LT [3, 111]. PC via AMPK activation reduced the ATP depletion thus attenuating the accumulation of glycolytic intermediates and lactate production during hepatic sustained ischemia [175]. The benefits of PC on oxidative stress could be explained by the induction of antioxidants, such as SOD and HSPs as well as by its effect on XDH/XOD [3, 111, 177]. PC reduced the accumulation of xanthine during ischemia and prevented the conversion of XDH to XOD, thus preventing the deleterious effect of this ROS generating system on liver [109,175]. It is possible that NFκB and p38 MAPK-regulated transcription factors (ATF-2 and MEF2C) might be responsible for inducing the expression of protective genes, including SOD. HSPs induced by PC might contribute to improve membrane potential and respiratory control in hepatic mitochondria, allowing a faster recovery of ATP on reoxygenation [111, 177]. The modulation of inflammatory response by hepatic PC has been also reported in steatotic livers undergoing warm or cold hepatic ischemia. PC reduces neutrophil accumulation, the generation of different cytokines and interleukins including TNF and IL-1 [109, 129, 175]. The benefits of PC were also observed on hepatic microcirculation by inhibiting the effects of different vasoconstrictor mediators such as ETs, thus ameliorating sinusoidal perfusion and microvascular dysfunction. The benefits of PC regulating cAMP, Ang II and adipocytokines such as adiponectin and RBP4 have been also reported in steatotic livers undergoing hepatic I/R [155]. PC, through PPARα inhibits adiponectin accumulation in steatotic livers and adiponectin-worsening effects on oxidative stress and hepatic injury in hepatic resections [129]. In LT PC, which increases RBP4 levels, reduced PPARγ levels and hepatic injury in steatotic livers [113]. PC increased the expression of Beclin-1 and LC-3, two pro-autophagic proteins, thus protecting against I/R injury in normal and fatty livers. The benefits of PC were also observed in reduced-size LT since reduced damage and improved liver regeneration. PC reduced oxidative stress and inhibited IL-1 through NO, thereby protecting against the injurious effects of IL-1 in reduced-size LT. In addition, by another pathway independent of

NO, PC induced HSP70 and HO-1. HO-1 protect against I/R injury and liver regeneration whereas the benefits resulting from HSP70 were mainly related to hepatocyte proliferation [178]

Since the effectiveness of PC was first described, numerous efforts have been made to find strategies capable of mimicking its beneficial effects. One of these strategies is known as heat shock preconditioning, in which the organ or the whole body is temporarily exposed to hyperthermia prior to hepatic ischemia. Chemical preconditioning with either doxorubicine, atrial natriuretic peptide or oxidants decreases hepatic injury in several experimental models of I/R. However, their possible clinical application seems limited owing to difficulties in implementing them in clinical practice, toxicity problems and the side-effects that have been identified [3,111].

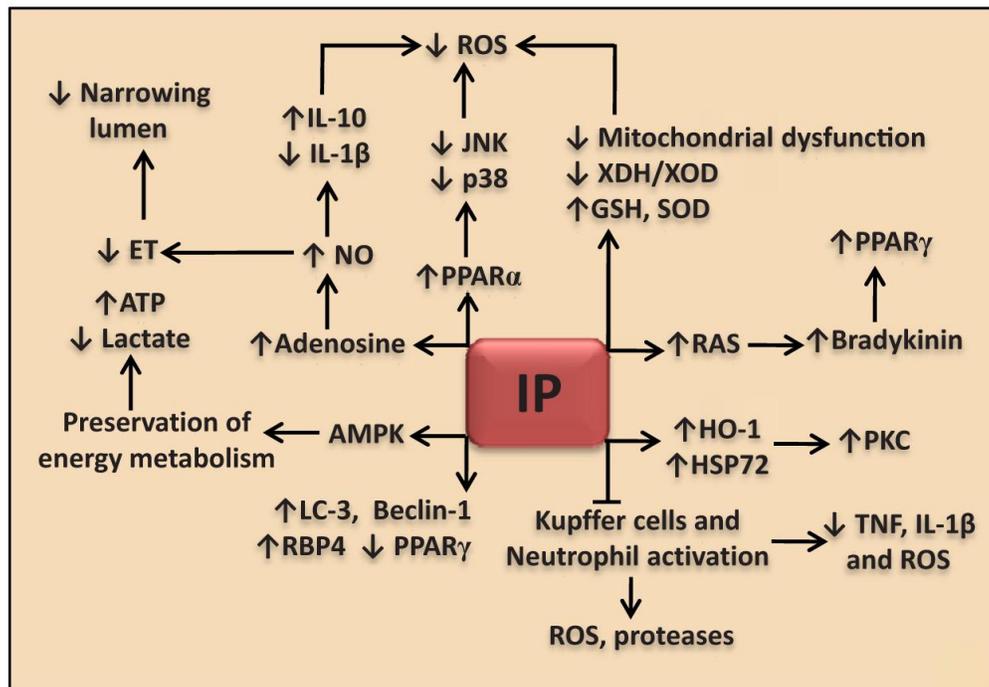


Figure 3. Mechanisms of Ischemic preconditioning. AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; ET, endothelin; GSH, glutathione; HO-1, heme oxygenase 1; HSP72, heat shock protein 72; IL, interleukin; JNK, c-Jun N-terminal kinase; LC-3, autophagic marker; NO, nitric oxide; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; RAS, renin-angiotensin system; RBP4, retinol binding protein 4; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF, tumor necrosis factor; XDH/XOD, xanthine/xanthine oxidase.

The benefits of PC observed in experimental models of hepatic warm and cold ischemia created the need for human trials of PC. To date, PC has been successfully applied in human liver resections in both steatotic and non-steatotic livers. The effectiveness of PC in hepatic surgery was first reported by Clavien et al., but unfortunately, in this study, it proved ineffective in elderly patients. It is well known that the impact of cold ischemia on organ function becomes even more significant as the age of the donor increases [1]. Recent research indicates that melatonin prevents oxidative stress and inflammatory response in hepatocytes

from elderly rats and this could improve the viability of liver grafts from elderly donors and increase the effectiveness of PC [179]. Prevention of post-hepatectomy liver insufficiency by PC, particularly in patients with cirrhotic or steatotic livers has also been demonstrated [180]. A clinical study by Koneru et al., [181] showed no effects of PC on cadaveric donor livers compared with controls. However, the study consisted of clamping the hepatic vessels for a period of 5 minutes, and as the authors concluded, that may be insufficient to obtain a beneficial effect from PC. Another clinical study carried out by Azoulay et al., [182] using the model of cadaveric whole LT showed that PC based on 10 minutes of ischemia was associated with better tolerance to ischemia. However, this was at the price of decreased early function. Jassem et al., [183] concluded that 10 minutes of preconditioning was effective to protect cadaveric donor allografts from cold ischemia, reduced inflammatory response and resulted in better graft function. Further randomized clinical studies are necessary to confirm whether PC is appropriate for LT in clinical practice. The potential applications of PC in human LT are numerous. PC also has the potential to increase the number of organs suitable for LT since it can improve the outcome for marginal grafts that would not otherwise have been transplanted. Its benefits to reduce the vulnerability of steatotic grafts to I/R injury have also been reported in different experimental studies of LT [109, 154]. Interestingly, the effectiveness of PC in clinical practice in major liver hepatectomy opens up new possibilities in LDLT, since the ischemia period is similar in both surgical procedures. Moreover, PC increases liver regeneration, the most critical aspect to be considered in LDLT [178]. In fact, a study published by Barrier et al., [184] has shown the benefits of PC in transplantation from living human liver donors.

## Recipient Selection

Recipient selection is a critical factor to be considered when deciding on whether to transplant a partial graft with the associated higher risk of failure or dysfunction. A balance must be struck between the risk of transplanting the graft and the risk that the patient will deteriorate on the waiting list. This dilemma can be framed in the context of whether one considers the best outcome for an individual recipient or the best use of the graft [85]. Also, the general condition of the liver recipient can have a major impact on graft function: the bad host effect. Thus, marginal donors and suboptimal recipients are a poor combination [1]. Generally, patients with lower MELD scores have better outcomes, especially with the use of marginal, living donors, or split grafts as they can tolerate initial graft dysfunction better. These grafts perform better in patients who can tolerate a bigger insult immediately following transplantation when compared with high risk recipients. However, by definition, those with higher scores are those more urgently in need of grafting, and despite the higher risk of graft dysfunction and failure, the benefit to the recipient may be greater [185].

## Conclusion

Due to the persistent shortage of organs, the increasing number of patients and the waiting time for LT, the use of marginal donors has been increasing in the recent years

although criteria for the use vary from center to center. However the most appropriate use of marginal livers continues to be debated. Some of the difficulties in defining a marginal liver donor may be related to the absence of prospective, randomized studies, an excess of univariate survival analysis, studies that include an insufficient number of marginal donors, an absence of evolutive studies and no consensus about uniform a defined cut-off points. A careful use of selected marginal liver grafts is a viable option for expanding the donor pool. While outcomes are inferior to results with optimal whole-liver grafts, the continued and increasing use of marginal grafts has led to decreased mortality on the waiting list.

Because of the correlation between organ quality and post-transplant outcome, strategies to improve graft quality could gain a particularly important role in the setting of marginal donors for LT in the future. Marginal livers are more susceptible to I/R injury compared with standard grafts. The deleterious effects on graft function seem additive with the presence of multiple marginal characteristics and can contribute to the etiology of I/R injury experienced by the organ. Besides controlling for short cold-ischemia times, strategies to reduce graft injury could improve post-transplant outcome and help salvage organs that would be discarded otherwise. Multiple methods are currently being investigated to minimize the effects of IR injury to allow the use of marginal organs, including anti-inflammatory approaches to attenuate cytokines, blockade of adhesion molecules, anti-apoptotic strategies, among others. Other strategies with preliminary clinical applications such as PC and other strategies that are still in the experimental stage, including synthetic allografts, and hepatic dialysis, will need to be developed. Hoped for but as yet unachieved developments in LT are hepatocyte transplantation, and liver-directed gene therapy and stem cell transplantation.

Until there are enough donors to meet the needs of the transplant waiting list, marginal donors may be a viable option to expand the donor pool. Further research is needed to identify better tests for evaluating donor organs, provide longer-term follow-up of recipients of higher-risk organs, and develop alternative means to fill the donor-organ shortfall. It is important to understand that these advances would require a leap in technical, logistical, and scientific expertise. The donor shortage in LT has led not only to surgical innovations to expand the donor pool but also to complex ethical issues surrounding patient selection, marginal organ donation, and liver donation, and future work needs to focus on optimising outcomes for patients while making the best use of the scarce donor resource.

## Acknowledgments

This research was supported by the Ministry of Economy and Competitiveness (project grant SAF2012-31238) Madrid, Spain. Jiménez-Castro M.B. is in receipt of a fellowship from the Sociedad Española de Transplante Hepatico (SETH Foundation) (Barcelona, Spain).

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