Published in final edited form as:

Am J Transplant. 2012 December ; 12(12): 3176–3183. doi:10.1111/j.1600-6143.2012.04288.x.

Liver Defatting: An Alternative Approach to Enable Steatotic Liver Transplantation

N. I. Nativ^a, T. J. Maguire^a, G. Yarmush^a, D. L. Brasaemle^b, S. D. Henry^c, J. V. Guarrera^c, F. Berthiaume^a, and M. L. Yarmush^{a,d,*}

^aDepartment of Biomedical Engineering, Rutgers University, Piscataway, NJ

^bDepartment of Nutritional Sciences, Rutgers University, New Brunswick, NJ

^cCenter for Liver Disease and Transplantation, Department of Surgery, Columbia University Medical Center, New York, NY

^dCenter for Engineering in Medicine/Surgical Services, Massachusetts General Hospital, Boston, MA

Abstract

Macrovesicular steatosis in greater than 30% of hepatocytes is a significant risk factor for primary graft nonfunction due to increased sensitivity to ischemia reperfusion (I/R) injury. The growing prevalence of hepatic steatosis due to the obesity epidemic, in conjunction with an aging population, may negatively impact the availability of suitable deceased liver donors. Some have suggested that metabolic interventions could decrease the fat content of liver grafts prior to transplantation. This concept has been successfully tested through nutritional supplementation in a few living donors. Utilization of deceased donor livers, however, requires defatting of explanted organs. Animal studies suggest that this can be accomplished by *ex vivo* warm perfusion in a time scale of a few hours. We estimate that this approach could significantly boost the size of the donor pool by increasing the utilization of steatotic livers. Here we review current knowledge on the mechanisms whereby excessive lipid storage and macrosteatosis exacerbate hepatic I/R injury, and possible approaches to address this problem, including *ex vivo* perfusion methods as well as metabolically induced defatting. We also discuss the challenges ahead that need to be addressed for clinical implementation.

Keywords

Defatting; fatty liver; ischemia-reperfusion (I/R); liver; machine perfusion; normothermic; steatosis; transplantation

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

The Prevalence of Hepatic Steatosis in the Donor Pool

Liver steatosis (fatty liver) is an early- and mild-stage abnormality within a broad spectrum of conditions that fall under the umbrella of nonalcoholic fatty liver disease (NAFLD) (1). It is a very common chronic liver-related abnormality in North America and is estimated to occur in more than 65% of individuals who are obese (defined as BMI >30) (2). Morphologically, steatosis is the accumulation of lipid droplets within hepatocytes. Microvesicular steatosis is defined as an accumulation of relatively small lipid droplets that do not displace the hepatocyte nuclei. Microsteatotic livers can be successfully transplanted even at high steatosis percentages (3–6). In contrast, macrovesicular steatosis, which is characterized by large lipid droplets that displace the nucleus to the cell periphery, is a significant risk factor for primary nonfunction posttransplantation in humans and is associated with increased morbidity and secondary injury, such as renal failure (3–6). A recent largescale analysis suggests that macrovesicular steatosis in more than 30% of hepatocytes decreases 1-year survival posttransplant (5). Therefore, transplantation centers typically shy away from using procured livers exhibiting >30% macrovesicular steatosis for orthotopic liver transplantation. Macrosteatotic livers below this cutoff may be used, however, if transplantation can be performed after a static cold storage period not exceeding 6 h (3–6). On the other hand, in the presence of additional risk factors, such as increasing donor age, macrosteatotic livers are more likely to be discarded (5). Given that obesity in the United States has surged from 19.8% to 27.2% in the last decade (7), it is expected that the proportion of macrosteatotic livers in the donor pool will increase. Another daunting trend is the increasing age of the general population, which will superpose on the effects of the obesity epidemic. As a result, without measures to salvage more macrosteatotic livers, we are facing a likely decrease in the number of eligible donors in the coming years.

Elevated Sensitivity of Steatotic Livers to I/R Injury

Evidence suggests that steatosis increases sensitivity of the liver to the stresses that are inherent to the liver transplantation procedure, more specifically cold ischemia and I/R-related events. *In vivo* and *in vitro* studies using animal models suggest that liver injury due to I/R is largely initiated within the liver parenchymal cells (8–10). Furthermore, there is evidence that the presence of excess lipids within hepatocytes exacerbates the I/R response. For example, one study has shown that microsteatotic hepatocytes in culture are more sensitive to I/R-mediated injury compared to their lean counterparts. The level of injury, measured by hepatic intracellular enzyme release as a cellular death marker, was correlated with the level of intracellular triglyceride (TG) content (9). Furthermore, by comparing mouse models of microsteatosis and macrosteatosis, Selzner et al. showed that macrosteatotic livers are more susceptible to I/R-mediated injury than microsteatotic livers with a similar TG content, clearly suggesting that besides the amount of TG stored, the presence of macrodroplets also exacerbates I/R sensitivity (8). We must also be cognizant of the fact that in human livers, the presence of steatosis is a well-described response to hepatocyte injury and could also be a marker of one or several predisposing conditions that may contribute to the increased sensitivity to I/R. Nonetheless, as discussed further below, I/R sensitivity of steatotic hepatocytes can be reversed by reducing stored fat (9), consistent

with the notion that excess lipid storage is independently a major cause of I/R hypersensitivity.

Mechanisms of Elevated Sensitivity of Steatotic Livers to I/R Injury

Several mechanisms have been proposed to explain the exacerbating effect of steatosis on I/R injury, although it should be pointed out that these studies do not generally attempt to distinguish between microsteatotic and macrosteatotic livers. Human as well as animal studies suggest that post-I/R, steatotic livers are subject to more lipid peroxidation (9,11–13) and more exuberant proinflammatory responses, including increased release of proinflammatory mediators such as tumor necrosis factor- α (TNF- α) (13,14), and increased neutrophil infiltration (15). In addition, animal models have shown that the increased cellular volume of steatotic hepatocytes leads to narrowed and tortuous microvessels in steatotic livers, consistent with reduced hepatic and sinusoidal blood flow postinjury compared to lean livers (8,12,15,16). The impaired sinusoidal blood flow is also consistent with reported hepatocyte mitochondrial dysfunction and decreased intrahepatic energy (adenosine triphosphate, ATP) levels in steatotic livers (9,12,16). Interestingly, an *in vivo* Zucker rat model of I/R suggests that livers containing micro- and macrosteatosis exhibit hepatocellular necrosis as the predominant form of cell death while lean livers mainly exhibit apoptosis (17). It is possible that reduced ATP levels in steatotic livers favored necrosis versus apoptosis because the latter is an ATP-requiring pathway. It was reported that inhibiting apoptosis pathways in lean livers undergoing I/R is effective in reducing the extent of injury, but not effective in the case of steatotic livers due to the difference in cell death mechanisms (17).

Experimental Approaches to Target I/R Injury Mechanisms in Fatty Livers

A variety of techniques have been tested to address one or more of the putative mechanisms that predispose steatotic livers to I/R injury in experimental animals. These approaches generally consist of using pharmacological agents or preconditioning methods to turn on protective pathways before subjecting the liver to I/R stress.

Pharmacological approaches

While many pharmacological agents have been tested in the context of liver transplantation, only few have been used on macrosteatotic livers (6). Some of these agents have been added to the cold storage preservation solution, and found to reduce I/R injury-related markers in reperfused Zucker rat steatotic livers (12). For example, carvedilol, a beta- and alpha-adrenergic blocker to treat ischemic heart, reduced hepatic death markers, vascular resistance and reactive oxygen species, as well as increased bile production and hepatic ATP levels postreperfusion (12). In a separate study aimed at reducing the increased levels of peroxidation observed in steatotic livers, obese Zucker rat livers containing micro and macrosteatosis were intravenously administered the antioxidant glutathione (GSH)-ester shortly prior to reperfusion in a surgically induced hepatic ischemia model. The treatment elevated intracellular levels of GSH and significantly reduced lipid peroxidation, I/R injury markers and hepatic death (11). In a similar animal model, Laurens et al. successfully reduced liver injury markers and enhanced the 15-day survival rate from 40% to 70% by

increasing hepatic ATP content via intravenous administration of Tacrolimus 24 h prior to surgically induced I/R injury (18). In a hypothermic I/R model where hepatic micro- and macrosteatosis were induced by feeding the rats a choline- and methionine-deficient diet (CMDD), administration of the GSH precursor N-acetylcysteine 15 min before liver recovering normalized GSH levels, reduced hepatic death markers and microcirculatory injury (15). In the same study, it was also shown that pretreatment with an antibody to intercellular adhesion molecule-1 (ICAM-1) prior to subjecting the graft to 60 min of warm ischemia inhibited neutrophil infiltration and reduced hepatic death markers (15). In sum, although a significant reduction of several I/R injury markers was generally reported in these studies, in most cases, the level of injury remained high above that of steatotic livers which did not undergo I/R injury, or lean livers (11,12,18). It is worth noting that none of these studies investigated the possibility of combining several pharmacological agents at once; therefore it is possible that doing so would lead to more dramatic decrease in sensitivity to I/R injury.

Ischemic preconditioning approach

A surgical method whereby the major blood vessels to the liver are clamped momentarily, known as “ischemic preconditioning”, has been shown to reduce lipid peroxidation, hepatic microcirculation failure and neutrophil accumulation after subsequent I/R injury when applied to microsteatotic and macrosteatotic livers (8). This treatment restored microcirculatory parameters to those observed in lean livers for both micro- and macrosteatotic livers, but had a more mitigated benefit on cell death markers. While cell death markers in microsteatotic livers returned to levels found in lean livers, macrosteatotic livers remained above baseline (8). The protective mechanism appears to involve nitric oxide (NO) (8,11). Ischemic preconditioning has been shown to protect human livers against a subsequent period of ischemia in patients undergoing hemihepatectomy. The analysis of a subgroup of patients with mild-to-moderate steatosis presented reduced serum levels of liver damage markers (aspartate/alanine aminotransferases) when preconditioned (19).

Heat shock preconditioning approach

Another experimental approach to alleviate I/R injury is heat shock preconditioning, which has been shown to preserve microcirculatory parameters (sinusoidal perfusion rate, sinusoidal diameter, minimal leukocyte-endothelial adhesion) and prevent microvascular perfusion failure after surgically induced I/R in livers from obese Zucker rats containing micro- and macrosteatosis (20). This study reported decreased oxidative stress (as measured by the oxidized/ reduced glutathione ratio) and liver damage markers such as hepatic intracellular enzyme release (20). Similarly, in a CMDD rat liver transplantation model containing micro and macrosteatosis, heat shock preconditioning dramatically improved the recipient 1 week posttransplant survival rate to that of lean liver recipients (21). Interestingly, there was a time window of efficacy ranging from 6 h to 24 h postheat shock, which correlated with the dynamics of heat shock protein (HSP) expression in liver, in particular HSP72 and heme oxygenase-1 (HO-1) (20,21). The protective mechanisms of heat shock preconditioning are not fully understood, but the utility of this approach warrants further studies that would enable a rational design of heat shock preconditioning regimens to improve effectiveness and practicality.

Defatting as an Alternative Way to Recondition Steatotic Livers

The methods described above focus on reducing one or more of the I/R injury-related events that are elevated in macrosteatotic livers. If excessive lipid storage is indeed a primary initiating event in the exacerbated response of macrosteatotic livers to I/R, a conceptually different approach would be to more directly address the initiating cause of I/R hypersensitivity in steatotic livers by 'defatting' livers prior to subjecting the grafts to I/R. The concept has been tested in humans, where a combination of a proteinrich (1000 kcal/day) diet, exercise (600 kcal/day) and fibrate drugs (bezafibrate 400 mg/day) for 2–8 weeks led to a threefold reduction in macrosteatosis (22,23). In a separate study, the consumption of omega-3 fatty acids for 1 month was used to decrease macrosteatosis in three candidates for living donor transplantation who had biopsy-proven hepatic macrosteatosis >30% prior to donation (22,23). In both studies, the 'defatting' process led to decreased hepatic macrosteatosis, normalized donor liver tests and successful transplantation (22,23). It should be pointed out that alterations in diet and exercise could have wider effects on the donor metabolic state; therefore, we cannot exclude the possibility that besides decreased steatosis, additional indirect effects may have impacted the performance of the donor livers.

Animal studies in which diet was used to modulate liver fat content, and studies in cultured hepatocytes incubated with different medium compositions have clearly shown that when fatty hepatocytes or livers are cleared of intracellular lipids, they recover the normal response to I/R similar to that of their lean counterparts (9,24). For example, in a rat model of CMDD-induced macrosteatosis, pretreating the donors with a diet to reduce the intrahepatic TG content for 3 days or more prior to transplantation increased recipient viability from 0% to greater than 75% (9). Another study reported that 48 h of treatment of ob/ob mice with the fatty acid synthase inhibitor cerulenin resulted in a shift from macro to microsteatosis and improved survival after I/R injury (24). In addition, treatment with cerulenin for 2, 4 or 7 days before liver procurement and transplantation increased recipient survival proportionally with treatment duration. Notably, cerulenin treatment increased liver ATP and downregulated the endogenous mitochondrial uncoupling protein 2, which may have also contributed to its beneficial effects (24). Taken together, these studies demonstrate the principle that the higher sensitivity of macrosteatotic livers to I/R injury can be reversed by defatting.

Ex Vivo Machine Perfusion to Defat Steatotic Livers

The studies mentioned above describe defatting strategies that are effective if implemented over a period of several days and may be relevant in the case of living donor liver transplantation. For the defatting approach to be useful on procured liver grafts, however, the kinetics of defatting will need to be accelerated to a time scale of hours, so that defatting can fit into the existing logistics of liver transplantation that typically involve procurement to transplantation within a maximum of 12 h (25,26). Although pretreatment of deceased donors prior to organ procurement is theoretically possible, there is likely more flexibility in the types and doses of agents that could be used during machine perfusion on procured macrosteatotic livers (27). There is encouraging evidence that defatting is possible within a

few hours via *ex vivo* normothermic machine perfusion of procured steatotic livers from Zucker rats (28).

Besides being a potential delivery platform for defatting agents, machine perfusion of livers has been shown to be superior to static cold storage in restoring graft viability prior to transplantation when carried out on lean, ischemic, or macrosteatotic explanted rat livers (13,27,29). For example, during a 1 hour normothermic perfusion of macrosteatotic livers procured from CMDD rats, cell death markers were reduced while liver function markers, including bile production, ammonia clearance, urea production and ATP levels, were significantly higher, compared to livers that underwent static cold storage (30). While normothermic machine perfusion has yet to be studied on human livers, a recently completed human phase 1 clinical trial at Columbia University reported several beneficial effects of hypothermic machine perfusion on procured human livers. Hypothermic machine perfusion for up to 7 h resulted in reduced early allograft dysfunction, biliary complications, vascular complications and decreased hospital stay length compared to static cold storage (25). Follow-up studies showed that machine perfusion reduced proinflammatory markers while increasing hepatic ATP in the recipient (26). However, machine perfusion of human macrosteatotic livers has not been reported.

So far, three animal experimental studies have reported macrosteatotic liver defatting using *ex vivo* perfusion (13,28,31). In a preliminary study using porcine livers, *ex vivo* normothermic perfusion for 48 h led to 50% reduction in lipid droplet size in perivenous hepatocytes to reach the size found in control lean livers (31). During a 60-h perfusion defatting process, liver function markers such as bile, urea and albumin production maintained levels similar to that in control lean livers (31). Similar techniques (6 h at 20°C or less) applied to micro- and macrosteatotic livers procured from obese Zucker rats showed significantly improved preservation compared to static cold storage as indicated by reduced cell death markers and elevated liver functionality such as bile production (13). While perfusion at 4°C and 8°C improved steatotic liver preservation compared to static cold storage, perfusion at the highest temperature examined, 20°C, was superior to that of lower temperatures (13). It is worth noting that histological examination of the perfused livers revealed a decrease in steatosis at 20°C, but not at 4°C or 8°C, which may have contributed to the superior graft preservation (13).

In a separate study aimed at accelerating the defatting kinetics to be within the clinically relevant time scale of several hours, livers obtained from obese Zucker rats were normothermic perfused *ex vivo* with a cocktail of agents which proved their defatting potency in a microsteatotic hepatocyte culture model (28). After only 3 h of perfusion, a 50% reduction in intracellular triglyceride content as well as reduction in lipid droplet size was observed, as indicated in Figure 1 (28). The rapid rate of defatting in perfused livers is surprising, since the same agents required several days to defat cultured microsteatotic hepatocytes (28). One fundamental difference is that the culture systems consist of hepatocytes only and are devoid of nonparenchymal cells present in liver, although the role of the latter in defatting is elusive (9,10). Another plausible explanation is that organ perfusion provides efficient transport of nutrients and other factors to cells, as well as

effective removal of waste and secreted products, thus speeding up the defatting process in comparison to static cell culture systems (28).

Strategies to Further Enhance Defatting

Normothermic machine perfusion

Clinical implementation of defatting via normothermic perfusion of steatotic liver grafts, even if successful in laboratory animal experiments, will have to overcome several logistical obstacles. Although the concept of machine perfusion is gaining clinical acceptance, all systems used to date have been with hypothermic rather than normothermic conditions (25,32). The data suggest that hypothermic machine perfusion was beneficial to nonsteatotic livers compared to simple cold storage (25,26). Because the processes of lipid oxidation and export are likely to be significantly slower at lower temperatures, decreasing temperature may not be desirable for defatting, although this question has not been investigated experimentally (29,33). On the other hand, it may be interesting to evaluate supraphysiological temperatures, as it may be possible to trigger heat shock preconditioning, which has been shown to be protective against I/R stress, as discussed previously (21). Nevertheless, once more evidence is available to support the use of hypothermic perfusion; it is likely that clinician investigators will be inclined to move forward into the realm of normothermic perfusion.

Normothermic perfusion systems will need to be further developed into portable perfusion devices that are equipped to handle the rigors of clinical use and transportation by ground and air, a challenging endeavor given their complexity. The reliability of a normothermic perfusion system will require a much higher threshold, since any flow stoppage due to machine failure—either unrecognized or not acted upon—would cause rapidly damaging warm ischemia. In contrast, the same problem in hypothermic perfusion would not be as damaging since such systems have a ‘backup’ static hypothermic mode, in which case, the result would be similar to simple cold storage, which is the current standard used for transplantation (25–27).

Normothermic perfusion may however provide major advantages that could mitigate these concerns. During normothermic perfusion, the liver is in a metabolically active state, which enables thorough evaluation of its function and thus suitability for transplantation well beyond what is possible when livers are stored using simple cold storage or cold perfusion. The benefits of normothermic perfusion as a tool to deliver novel resuscitative interventions will likely result in researchers continuing to push the envelope in developing normothermic techniques applicable to clinical setting (27).

Optimizing the perfusion solution

Ultimately, the goal of liver defatting is to rapidly decrease the proportion of macrosteatotic hepatocytes, while maintaining high viability and functionality. Steatosis is the result of an imbalance between TG synthesis and breakdown processes in hepatocytes (1,2,9,28). Therefore, to achieve significant defatting, the protocol of choice should shift this balance towards more efficient TG breakdown (lipolysis) and excretion of related byproducts, as well as minimizing TG synthesis (9,28,31,34). The pathways responsible for lipid

metabolism are well known; however, there is much work to be done on how best to modulate this metabolism using cocktails of agents in order to achieve rapid defatting without adversely affecting viability and other critical liver functions.

There is a considerable body of literature on transcription factors that regulate lipid metabolism in liver; however, transcriptional regulation typically has a response lag time of greater than 6 h. Thus, compounds that target such pathways will be minimally effective over the time scale of *ex vivo* perfusion. Rather, agents that mediate their effects via posttranslational mechanisms, including signaling effectors, metabolic substrates and cofactors, should be considered for this approach. The major pathways that control TG storage, as well as defatting agents which have been tested to decrease TG storage in a short timeframe are summarized in Figure 2 and briefly discussed here. The control of lipolysis requires cooperation between perilipins, which are proteins associated with the surfaces of lipid droplets where TG is stored, and lipases, which cleave TGs into diacylglycerol, monoacylglycerol, FFAs and glycerol (28,34–36). Indirect activation of protein kinase A by compounds such as forskolin increases phosphorylation of perilipin 5 on the surfaces of lipid droplets and promotes lipolysis (28,36,37). The lipolysis products will readily reform TG unless they are further metabolized and/or excreted from the hepatocytes. This is achieved, in part, by reesterification of lipolysis products into TG to be packaged in VLDL particles, which are then secreted from the hepatocytes (34). The addition of amino acids and choline has been shown to promote the synthesis of apolipoprotein B and phospholipids, respectively, both critical for VLDL assembly and to reduce steatosis (28,38). Another approach to promote defatting is to increase the transport of FFAs to mitochondria where they undergo β -oxidation to generate ATP and CO₂, as well as ketone bodies that are excreted from the liver (28,39). A rate-limiting step in β -oxidation is the transport of FFAs from the cytoplasm into the mitochondria, which requires the conjugation of acyl-coA with L-carnitine by carnitine palmitoyltransferase-1 (CPT-1) located on the outer mitochondrial membrane (40). *In vivo* and *in vitro* studies have shown that supplementation of L-carnitine in the diet or cell culture medium promotes β -oxidation and a reduction in hepatic TG (40).

Targeting multiple routes simultaneously, as illustrated in Figure 2, may provide the most effective approach, but rational design of defatting protocols will require a better understanding of the potential interactions among the relevant metabolic pathways in macrosteatotic hepatocytes. This would be greatly facilitated if a suitable cell culture model of hepatic macrosteatosis were available to perform both screening and mechanistic studies in an efficient manner, prior to testing in actual livers. Although some steatotic hepatocyte culture systems have been described in the literature, they all exhibited microsteatotic, and not macrosteatotic, features (9,28).

Ultimately, surgeons will require proof that defatted livers are indeed similar to normal lean livers before this approach will gain wide acceptance. It will therefore be important to assess the short- and long-term functionality of steatotic livers for which TG content has been dramatically reduced by rapid normothermic *ex vivo* perfusion defatting in a relatively short period of time. While many challenges need to be overcome, liver defatting is a potentially promising approach to reduce the sensitivity of macrosteatotic livers to I/R injury, and a new

modality that may enable the successful recovery of a large number of livers that would otherwise be discarded.

Acknowledgments

This work was partially supported by NIH grant R01DK059766. N.I.N. and G.Y. were supported by an NIH-funded Biotechnology Training Fellowship.

Abbreviations

ATP	adenosine triphosphate
CMDD	choline- and methionine-deficient diet
CPT-1	carnitine palmitoyltransferase-1
FFA	free fatty acid
GSH	glutathione
HO-1	heme oxygenase-1
HSP	heat shock protein
ICAM-1	intercellular adhesion molecule-1
I/R	ischemia reperfusion
NAFLD	nonalcoholic fatty liver disease
NO	nitric oxide
TG	triglyceride
TNF-alpha	tumor necrosis factor-alpha.

References

1. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: Old questions and new insights. *Science*. 2011; 332:1519–1523. [PubMed: 21700865]
2. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology*. 2010; 51:679–689. [PubMed: 20041406]
3. de Graaf EL, Kench J, Dilworth P, et al. Grade of deceased donor liver macrovesicular steatosis impacts graft and recipient outcomes more than the donor risk index. *J Gastroenterol Hepatol*. 2012; 27:540–546. [PubMed: 21777274]
4. Perez-Daga JA, Santoyo J, Suárez MA, et al. Influence of degree of hepatic steatosis on graft function and postoperative complications of liver transplantation. *Transplant Proc*. 2006; 38:2468–2470. [PubMed: 17097969]
5. Spitzer AL, Lao OB, Dick AA, et al. The biopsied donor liver: Incorporating macrosteatosis into high-risk donor assessment. *Liver Transplant*. 2010; 16:874–884.
6. Guarrera JV, Karim NA. Liver preservation: Is there anything new yet? *Curr Opin Organ Transplant*. 2008; 13:148–154. [PubMed: 18685295]
7. Fryar, CD.; Ogden, CL.; Carroll, MD. Prevalence of overweight, obesity, and extreme obesity among adults: United States, Trends 1960–1962 through 2007–2008. Hyattsville, MD: Centers for Disease Control and Prevention; 2010. Available at: http://www.cdc.gov/nchs/data/hestat/obesity_adult_09_10/obesity_adult_09_10.html. [Accessed April 18, 2012]

8. Selzner N, Selzner M, Jochum W, Amann-Vesti B, Graf R, Clavien P-A. Mouse livers with macrosteatosis are more susceptible to normothermic ischemic injury than those with microsteatosis. *J Hepatol.* 2006; 44:694–701. [PubMed: 16229921]
9. Berthiaume F, Barbe L, Mokuno Y, MacDonald AD, Jindal R, Yarmush ML. Steatosis reversibly increases hepatocyte sensitivity to hypoxia-reoxygenation injury. *J Surg Res.* 2009; 152:54–60. [PubMed: 18599084]
10. Taneja C, Prescott L, Koneru B. Critical preservation injury in rat fatty liver is to hepatocytes, not sinusoidal lining cells 1. *Transplantation.* 1998; 65:167–172. [PubMed: 9458009]
11. Serafin A, Roselló-Catafau J, Prats N, Xaus C, Gelpi E, Peralta C. Ischemic preconditioning increases the tolerance of fatty liver to hepatic ischemia-reperfusion injury in the rat. *Am J Pathol.* 2002; 161:587–601. [PubMed: 12163383]
12. Ben Mosbah I, Roselló-Catafau J, Alfany-Fernandez I, et al. Addition of carvedilol to University Wisconsin solution improves rat steatotic and nonsteatotic liver preservation. *Liver Transplant.* 2010; 16:163–171.
13. Vairetti M, Ferrigno A, Carlucci F, et al. Subnormothermic machine perfusion protects steatotic livers against preservation injury: A potential for donor pool increase? *Liver Transplant.* 2009; 15:20–29.
14. Selzner M, Clavien P-A. Fatty liver in liver transplantation and surgery. *Semin Liver Dis.* 2001; 21:105,14.
15. Nakano H, Nagasaki H, Barama A, et al. The effects of N-acetylcysteine and anti-intercellular adhesion molecule-1 monoclonal antibody against ischemia-reperfusion injury of the rat steatotic liver produced by a choline-methionine-deficient diet. *Hepatology.* 1997; 26:670–678. [PubMed: 9303498]
16. Sun C-K, Zhang X-Y, Zimmermann A, Davis G, Wheatley AM. Effect of ischemia-reperfusion injury on the microcirculation of the steatotic liver of the Zucker Rat1. *Transplantation.* 2001; 72:1625–1631. [PubMed: 11726821]
17. Selzner M, Rüdiger HA, Sindram D, Madden J, Clavien P-A. Mechanisms of ischemic injury are different in the steatotic and normal rat liver. *Hepatology.* 2000; 32:1280–1288. [PubMed: 11093735]
18. Laurens M, Scozzari G, Patrono D, et al. Warm ischemia-reperfusion injury is decreased by tacrolimus in steatotic rat liver. *Liver Transplant.* 2006; 12:217–225.
19. Pierre-Alain Clavien SY, Sindram D, Rex CB. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. *Ann Surg.* 2000; 232:155–162. [PubMed: 10903590]
20. Yamagami K, Enders G, Schauer RJ, et al. Heat-shock preconditioning protects fatty livers in genetically obese Zucker rats from microvascular perfusion failure after ischemia reperfusion. *Transplant Int.* 2003; 16:456–463.
21. Mokuno Y, Berthiaume F, Tompkins RG, Balis UJ, Yarmush ML. Technique for expanding the donor liver pool: Heat shock preconditioning in a rat fatty liver model. *Liver Transplant.* 2004; 10:264–272.
22. Nakamuta M, Morizono S, Soejima Y, et al. Short-term intensive treatment for donors with hepatic steatosis in living-donor liver transplantation. *Transplantation.* 2005; 80:608–612. [PubMed: 16177634]
23. Clavien PA, Oberkofler CE, Raptis DA, Lehmann K, Ricken-bacher A, El-Badry AM. What is critical for liver surgery and partial liver transplantation: Size or quality? *Hepatology.* 2010; 52:715–729. [PubMed: 20683967]
24. Chavin KD, Fiorini RN, Shafizadeh S, et al. Fatty acid synthase blockade protects steatotic livers from warm ischemia reperfusion injury and transplantation. *Am J Transplant.* 2004; 4:1440–1447. [PubMed: 15307831]
25. Guarrera JV, Henry SD, Samstein B, et al. Hypothermic machine preservation in human liver transplantation: The first clinical series. *Am J Transplant.* 2010; 10:372–381. [PubMed: 19958323]
26. Guarrera JV, Henry SD, Chen SWC, et al. Hypothermic machine preservation attenuates ischemia/reperfusion markers after liver transplantation: Preliminary results. *J Surg Res.* 2011; 167:e365–e373. [PubMed: 20451921]

27. Vogel T, Brockmann JG, Coussios C, Friend PJ. The role of normothermic extracorporeal perfusion in minimizing ischemia reperfusion injury. *Transplant Rev.* 2012; 26:156–162.
28. Nagrah D, Xu H, Tanimura Y, et al. Metabolic preconditioning of donor organs: Defatting fatty livers by normothermic perfusion ex vivo. *Metab Eng.* 2009; 11:274–283. [PubMed: 19508897]
29. Tolboom H, Izamis M-L, Sharma N, et al. Subnormothermic machine perfusion at both 20°C and 30°C recovers ischemic rat livers for successful transplantation. *J Surg Res.* 2012; 175:149–156. [PubMed: 21550058]
30. Bessems M, Doorschodt BM, Kolkert JLP, et al. Preservation of steatotic livers: A comparison between cold storage and machine perfusion preservation. *Liver Transplant.* 2007; 13:497–504.
31. Jamieson RW, Zilvetti M, Roy D, et al. Hepatic steatosis and normothermic perfusion—preliminary experiments in a porcine model. *Transplantation.* 2011; 92:289–295. [PubMed: 21681143]
32. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2009; 360:7–19. [PubMed: 19118301]
33. Dutta A, Vats P, Singh V, Sharma Y, Singh S, Singh S. Impairment of mitochondrial β -oxidation in rats under cold-hypoxic environment. *Int J Biometeorol.* 2009; 53:397–407. [PubMed: 19396472]
34. Lankester DL, Brown AM, Zammit VA. Use of cytosolic triacylglycerol hydrolysis products and of exogenous fatty acid for the synthesis of triacylglycerol secreted by cultured rat hepatocytes. *J Lipid Res.* 1998; 39:1889–1895. [PubMed: 9741702]
35. Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem.* 1997; 272:27218–27223. [PubMed: 9341166]
36. Wang H, Bell M, Sreenivasan U, et al. Unique regulation of adipose triglyceride lipase (ATGL) by perilipin 5, a lipid droplet-associated protein. *J Biol Chem.* 2011; 286:15707–15715. [PubMed: 21393244]
37. Wolins NE, Quaynor BK, Skinner JR, et al. OXPAT/PAT-1 Is a PPAR-induced lipid droplet protein that promotes fatty acid utilization. *Diabetes.* 2006; 55:3418–3428. [PubMed: 17130488]
38. Raubenheimer PJ, Nyirenda MJ, Walker BR. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. *Diabetes.* 2006; 55:2015–2020. [PubMed: 16804070]
39. Chan C, Berthiaume F, Lee K, Yarmush ML. Metabolic flux analysis of hepatocyte function in hormone- and amino acid-supplemented plasma. *Metab Eng.* 2003; 5:1–15. [PubMed: 12749840]
40. Murosaki S, Lee TR, Muroyama K, et al. A combination of caffeine, arginine, soy isoflavones, and L-carnitine enhances both lipolysis and fatty acid oxidation in 3T3-L1 and HepG2 cells in vitro and in KK mice in vivo. *J Nutr.* 2007; 137:2252–2257. [PubMed: 17885007]

Figure 1. Histological appearance of defatted steatotic livers procured from 14- to 15-week-old Zucker rats

Livers were examined before and after 3 h of normothermic perfusion in a recirculating perfusion system. (A, B) Normal lean and fatty livers before perfusion. (C) Fatty liver after perfusion with control vehicle solution. (D) Fatty liver after perfusion with a solution containing a cocktail of defatting agents. Right hand panels are higher magnifications of the left hand panels. Reproduced with permission from reference (28).

Figure 2. TG metabolism in steatotic hepatocytes

Lipids are primarily stored in the form of TG inside lipid droplets which are coated with perilipins. Perilipins control access of the stored TG to cytosolic lipases, which liberate free fatty acids (FFA). FFAs can then undergo oxidation through mitochondrial β -oxidation. FFA and MG translocate to the ER, where they can be reesterified to TG and packaged into VLDL, which is then secreted out of the cells. Major lipid metabolic pathways, including those involved in the removal of TG from steatotic hepatocytes are illustrated. Addition of potential defatting agents that target some of these pathways, such as forskolin, L-carnitine, amino acids and choline, to the perfusate are shown here and discussed further in the text. The dashed arrow represents indirect activation of protein kinase A by forskolin. TG, triglyceride; FFA, Free fatty acid; DG, diacylglycerol; MG, monoacylglycerol; VLDL, very low density lipoprotein; CPT-1, carnitine palmitoyltransferase-1; ER, endoplasmic reticulum; ATP, adenosine triphosphate.