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Shawn D. St Peter
Children's Mercy Hospital
Ashok Handa
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Hepatic Steatosis and Its Relationship to Transplantation
Charles J. Imber, Shawn D. St. Peter, Ashok Handa, and Peter J. Friend

Fatty infiltration of the liver is common in the brain-dead donor population and has a strong correlation with primary nonfunction after cold preservation, a condition that is catastrophic to liver transplant recipients. This literature review examines factors associated with the development, diagnosis, quantification, and clinical management of this difficult condition. (Liver Transpl 2002; 8:415-423.)

Biochemical liver function test results frequently fail to identify a liver with even a considerable degree of steatosis. However, such a liver is at substantial risk for primary nonfunction (PNF), or at least a period of very poor function after reperfusion. In either case, the patient is placed at considerable risk. With the shortage of donor organs, steatotic livers are used increasingly for transplantation, and there is a clear need for reliable and objective means to assess a potential donor liver before transplantation. Audit on a national scale is required so that many of the contentious issues regarding the transplantation of steatotic organs can be assessed and improved, maximizing the effective use of a scarce resource and allowing established guidelines based on sound experimental or clinical evidence to be drawn up. It also is important to understand mechanisms by which fat deposition occurs and has such a deleterious effect on the outcome of transplantation.

Methods
A systematic review of the literature on steatosis was performed using the MEDLINE database to ascertain current thinking on fatty change and its relevance in transplant surgery.

Results and Conclusion
A wide variety of clinical practice based mainly on individual preference and anecdotal evidence exists. Histological assessment of donor biopsy specimens requires standardization if it is to be universally accepted, although computed tomography (CT) would provide a more accurate method despite obvious logistic constraints. Normothermic machine perfusion may prevent risks associated with cold preservation, as well as provide a method of viability assessment, and is worthy of future research.

Review
Pathological Evaluation
The term fatty liver identifies a liver in which lipid, mainly triglyceride, accounts for more than 5% of liver wet weight. Size of the fat vacuole determines the category in which fatty change should be classified:

Macrovesicular steatosis is the most usual form in humans, and in this condition, the vacuole (mainly triglycerides) takes up the majority of the hepatocyte cytoplasm and the nucleus is squeezed to the periphery. In the absence of other liver lesions, it is by itself a relatively benign condition. It may be associated with a mild increase in serum transaminase levels, an enlarged liver on physical examination, and hyperreflective hepatomegaly on ultrasound. The most frequent causes include alcohol abuse, diabetes, obesity, and some dyslipidemias, and it can occur in combination with microvesicular change.

Microvesicular steatosis, in which more than 90% of vacuoles observed are smaller than the hepatocyte nucleus, is typically associated with acute fatty liver of pregnancy and Reye's syndrome and is known to progress to fulminant and subfulminant hepatic failure. More rare causes include high doses of intravenously administered tetracycline and valproic acid; indirect injury to the liver, such as in septic or low-flow states; inherited metabolic disorders; and in association with the cholestasis of starvation or extended use of total parenteral nutrition.

At the outset, lipid accumulates in a microvesicu-
lar form within the cytoplasm of liver cells, predominantly in the perivenular (centrilobular) zone. It reflects interference with lipid exit from the liver through reduced synthesis of the apoprotein of very low-density lipoproteins. Mitochondrial injury also is reported to reduce fatty acid oxidation; a block in the beta oxidation of fatty acids in Reye’s syndrome has been identified. This form of fat deposition is believed to represent a response to an acute viral, toxic, or nutritional disturbance as opposed to macromesicular change, which represents a more chronic insult (e.g., alcohol or diabetes).

Fatty liver can evolve into its inflammatory counterpart, steatohepatitis, because of excess alcohol consumption, or even in the nondrinker, so-called nonalcoholic steatohepatitis (NASH) syndrome. This is diagnosed in the presence of (1) a biopsy specimen showing moderate to gross macrovesicular steatosis with inflammation with or without Mallory’s hyaline bodies, fibrosis, or cirrhosis; (2) convincing evidence of negligible alcohol consumption; and (3) absence of antibodies to hepatitis C virus, evidence of ongoing hepatitis B virus infection, markers of autoimmunity, and Wilson’s disease.

The usually slow progression of fatty change to NASH is held to be the precursor of cryptogenic cirrhosis.

Mechanism and Stimulus for Fat Deposition
Accumulation of triglycerides within hepatocytes is the hallmark of this group of diseases. This may result from defects in any one of the events in the sequence from fatty acid entry to lipoprotein exit: (1) excessive entry of free fatty acids into the liver, seen in starvation; (2) enhanced fatty acid synthesis and decreased fatty acid oxidation; (3) increased esterification of fatty acids to triglycerides because of an increase in alpha-glycerophosphate, believed to be one effect of alcohol poisoning; and (4) decreased apoprotein synthesis associated with carbon tetrachloride poisoning.

Any etiologic agent that causes a liver injury may induce fatty change, acting at more than one location within the complex process of fat metabolism. Alcohol, the most common cause of fatty change within industrialized countries, increases free fatty acid synthesis, diminishes triglyceride use, decreases fatty acid oxidation, blocks lipoprotein excretion, and enhances lipolysis.

Incidence
A wide disparity exists in the reported prevalence of fatty metamorphosis in livers.

First, a clear distinction must be made between a series of autopsy specimens and biopsy specimens procured from brain-dead organ donors because the latter group is known to develop a predisposition to steatosis independently.

Hornboll and Olsen conducted an adult autopsy study based on hospitalized patients and found moderate to severe fatty change (>30% of hepatocytes involved) in 11% of their subjects, with a strong association between obesity and steatosis. This is similar to rates of steatosis found in potential living related liver donors (6% to 10%), who should represent a relatively healthy nonbiased population. Far greater rates of fatty change were found in studies specifically dealing with victims of traumatic deaths. In a study investigating 503 consecutive victims of fatal traffic accidents, the corresponding figure was 24%. Similarly, using Oil red O stain for fat in livers of 21 children who had died in traumatic accidents with no preexisting comorbidity, all had some degree of steatosis and 30% showed moderate to severe microvesicular change. These very high levels of steatosis may reflect relative hypoperfusion of the liver in a shocked patient after trauma.

The prevalence of fatty livers among cadaveric brain-dead adult and child donors ranges from 13% to 26% when biopsy specimens are stained with hematoxylin and eosin (H&E). This large discrepancy among centers may be caused by variations between donor pools and definitions of fatty change. Far greater levels of steatosis have been identified using specific fat stains by other groups. Markin et al, using Oil red O, found steatosis in 51% of 187 livers. However, the investigators dismissed this figure because, in their opinion, this staining technique was unreliable for fatty change, staining sinusoids as well as vacuoles. Another series of 83 consecutive liver wedge biopsy specimens obtained at liver procurement, on the backbench, and after reperfusion showed greater than 30% steatosis in 49% of sections stained with toluidine blue solution (TBS).

Experimental Models of Fatty Liver
Zucker rats with genetic mutation overeat compared with their lean cohorts and develop obesity, nonketotic hyperglycemia, and steatosis of the liver caused by excessive synthesis and storage of triglyceride. On histological examination, their livers show macrovesicular and microvesicular steatosis, but no necrosis or inflammation.

Fatty infiltration also can be induced by manipulat-
ing the diet. Deficiencies in choline lead to a reduction in phosphatidylcholine, an essential component of very low-density lipoprotein responsible for the transport of triglyceride out of the cell. After 28 days of a choline methionine–deficient diet, rat livers developed fat accumulation with no evidence of necrosis, whereas after 42 days, fatty changes, as well as hepatocyte necrosis, were present. Gas chromatography in this model showed that fatty acid content reached a maximum by day 14 and remained relatively constant for an additional 28 days, with greater than 66% of hepatocytes involved on H&E staining.

Using a rat model of diet-induced fatty liver, Teramoto et al showed no correlation with transplantation outcome and increasing fat (>66%) unless preoperative hepatocyte necrosis was evident histologically or preservation times exceeded 12 hours. This is in contrast to the study of Hayashi et al, who reported 100% mortality (eight of eight rats) using the same model, inducing 30% to 60% fat infiltration after 9 hours of cold University of Wisconsin solution preservation.

The intragastric alcohol infusion rat model has been used in various laboratories to study various aspects of alcoholic liver disease, including steatosis. This model is valuable because diet and ethanol intake are totally under the control of the investigator. It has been found that ethanol is not toxic to the liver unless the accompanying diet is rich in polyunsaturated fatty acids; the latter aid in the generation of free radicals that attack lipids to form lipid peroxides.

Large-animal models include a high-fat (51%) choline-deficient diet, used in canine studies of fatty infiltration that bears a closer resemblance to the obesity-associated steatosis seen in humans. Macrophages are totally involved with length of exposure. Finally, pigs administered 1 g/kg of alcohol (100%) as a 1:3 mixture in water through an orogastric tube for 16 days developed steatosis (degree not specified), with no associated elevation in plasma γ-glutamyltransferase or aspartate aminotransferase levels.

Different etiologic agents cause different patterns of fatty infiltration, which may have important implications in both clinical and experimental settings. In human liver, for example, fatty infiltration is central lobular in diabetes and obesity and predominantly so in alcoholism, but periportal in protein-calorie malnutrition. In animal models, the low–choline methionine diet produces periportal infiltration, whereas the cholesterol model used by Seifalian et al induces central lobular change. These regional differences may reflect zonal biochemical variations within liver sinusoids.

**Mechanisms Linking Fatty Change to PNF**

Hepatic steatosis may develop without clinical or biochemical evidence of liver disease. However, after cold preservation, these organs show an increased susceptibility to ischemia-reperfusion injury and thus a high incidence of PNF after transplantation. Mechanisms behind this are still not completely understood.

Experimental studies of steatotic animal models showed an inverse correlation between degree of steatosis and sinusoidal blood flow, believed to be caused by ballooned hepatocytes containing fat droplets that compress and distort the sinusoidal lumen and increase intrahepatic portal resistance. This in turn leads to relative ischemia of fatty hepatocytes, shown to have increased sensitivity to anoxia in culture and thus exaggerating the susceptibility of these organs to preservation-reperfusion injury. Seifalian et al also note that this shunting of blood from the hepatic microcirculation may be responsible for impaired perfusion of an organ with cold preservation solution, leading to more damage during the preservation process.

Inefficient anaerobic metabolism during warm ischemia and cold preservation, with the depletion of high-energy phosphates and buildup of lactic acid, is believed to be one of the major mechanisms contributing to reperfusion injury. The energy level within the hepatocyte after the period of preservation has been shown to correlate with eventual outcome after transplantation.

Several investigators have shown abnormal adenosine triphosphate production within the mitochondria of steatotic livers. This is believed to be caused by the accumulation of nonesterified fatty acids and has been postulated as a possible mechanism for the increased sensitivity of fatty livers to ischemic injury.

Some postulate that the critical injury is to the sinusoidal lining cell, with an alteration in plasma membrane fluidity and subsequent blood cell adhesion and Kupffer’s cell activation. Others suggest that fat-laden hepatocytes undergo changes, including solidification during cold injury, and are responsible for increased sensitivity to reperfusion injury, releasing fatty globules that can disrupt the sinusoid microcirculation. The massive accumulation of lipids released from hepatocytes within sinusoids after transplantation, mimicking peliosis, has been termed lipopeliosis.
tion, with free radical formation, thus causing additional cellular damage. Two grossly fatty human livers transplanted and subsequently removed within 3 days because of PNF showed extracellular fat globules, with associated distortion of sinusoids on histological appraisal. However, using a methionine-deficient diet in rats, Teramato et al refuted this theory by showing a relative increase in unsaturated fatty acid, which they argued would render cells more resistant to solidification during cold storage.

It also has been shown in an experimental animal model that steatotic livers are more susceptible to injury during subsequent periods of warm ischemia. Disruption of the sinusoidal microvasculature again is postulated to be the underlying mechanism. In view of the increased sensitivity of steatotic livers to both warm and cold ischemia, the logistic difficulty placed on transplantation of this group of organs increases, with both times required to be as short as possible, thereby minimizing the risk for PNF. These constraints, together with recipient factors that may prejudice outcome, need to be taken into account when a decision is made to use a steatotic organ.

Assessment of Donor Livers

Attempts at practical assessment of the degree of steatosis have not proved reliable. An experienced transplant surgeon is said to be able to estimate the degree of fatty change within a liver at the donor retrieval. However, in one series, 66% of steatotic livers were described as normal on macroscopic appearance by a surgeon, with predictive values of 71%, 46%, and 17% for massive, moderate, and mild change, respectively. In another study, 38% of livers judged normal macroscopically showed fatty change on histopathologic appraisal. Certainly, severe steatosis can be identified by yellow discoloration after flushing, rounded edges, and a greasy firm texture. However, this highly subjective test is less sensitive with mild to moderate steatosis.

If a scientific approach regarding the incorporation of fatty livers in clinical practice is to be achieved, a consensus regarding classification of the degree of steatosis needs to be established. This may require standardization of biopsy procedures, material preservation, and staining techniques to achieve a uniform mechanism of assessing donor livers. Ploeg et al suggested classification of fatty change as mild (<30% of visualized hepatocytes involved), moderate (30% to 60%), and severe (>60%), a system that is approximately applied by most centers.

In the early 1990s, four large studies examined the relationship of fatty change to PNF (Table 1). The largest of these assessed 390 frozen-section biopsy specimens and found that 13% of grafts showing greater than 30% steatosis had PNF compared with 2.5% of nonsteatotic grafts. Progressive deterioration in graft survival was observed from mild to massive steatosis; it was concluded that grafts with severe steatosis should be discarded, and those with moderate change should be evaluated in conjunction with other criteria, such as condition of the recipient and availability of organs at that time. The institution involved, in line with most others worldwide, found no contraindication to transplanting livers with minimal change. This concurs with the findings of Ploeg et al, who found PNF rates as high as 80% in severely steatotic organs, but more worryingly, initial poor function rates as high as 30% in moderately steatotic livers. A routine, mandatory, donor liver biopsy before flush out and cold storage was recommended by the investigators of this study. By applying this edict and excluding livers with severe steatosis (>45% in this study), Markin et al were able to reduce PNF rates from 8.5% to 1.4% after transplantation. From these studies, it is concluded that

Is Microvesicular Steatosis a Risk Factor for PNF?

In the cadaveric donor setting, Fishbein et al reviewed 426 transplants and identified 40 cases containing moderate to severe microvesicular steatosis (>30% steatosis). Donor obesity (42%) and traumatic death (68%) were the most commonly associated risk factors. In this study, the incidence of PNF and poor early graft function in this group of livers was 5% and 10%, respectively; there was no significant difference in these values from normal livers. It was concluded that high-grade (>60%) microsteatosis should not be considered a contraindication to the use of a liver for subsequent transplantation. Conversely, Yoong et al described a retrospective analysis of a series of 116 liver retransplantations in which microvesicular steatosis was found in 73 patients (63%) and quantified histologically. They found that severe change affecting greater than 66% of hepatocytes was associated with a significantly worse outcome in this group, with a reduced overall 1-year graft survival rate of 20% compared with 57% in the nonsevere group. The latter study included a sicker subpopulation of patients receiving their second, third, or fourth grafts, which may in part explain the incongruous conclusions reached.
the presence of severe steatosis is an absolute contraindication to the use of that organ for transplantation. Most surgeons would consider moderate steatosis a risk factor for eventual graft loss and only use the organ if other risk factors, such as prolonged preservation time and poor health of the recipient, were not present. It has been shown to have an association with primary graft dysfunction, which in itself has been shown to have a detrimental effect on eventual graft outcome by some investigators.

### Living Related Transplantation

Hayashi et al. investigated the effect of steatosis on living related donor livers. They showed that early graft function was similar in mild and moderate steatosis, but severe disease was significantly associated with poor function and outcome. No cases of PNF were evident in this group because of the short cold ischemia times possible in living related transplantation. Interestingly, they were able to include five donor candidates in

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Abbreviations: ORO, Oil red O; SIII, Sudan III; NS, not stated. *One-month survival, non-PNF rate.
whom diet and exercise therapy had resulted in improved liver function test results and degree of fatty infiltration, although this was not quantified in the report. In contrast to other published work in which hepatectomy was performed in the presence of steatosis, there was no increased mortality or morbidity in their donors.42-43

Methods of Quantifying Fatty Change

Staining. Some groups describe taking needle cores, whereas others use wedge biopsy specimens ranging from one to five randomly cut sections.9 The method of staining varies between H&E, in which the approximate area of nonstaining vacuolation is described as fatty change, and specific fat stains, such as Oil red O or Sudan IV, in which a positive color change is required. These specific stains are not available in an emergency setting.

Problems have been identified with specific fat stains. Oil red O staining quality and amount of staining of a specific section has been shown to be highly operator dependent; the rinse step confers most variability to the procedure. High false-positive rates caused by sinusoidal staining also have been described, especially after preservation in University of Wisconsin solution.9

Urena et al7 compared four staining techniques: Sudan III, TBS, frozen H&E, and deparaffinated H&E. They showed that TBS staining using thin sections embedded in resin provides the greatest sensitivity because fat does not dissolve during processing and small vacuoles are not masked by overlying or underlying cytoplasmic content. The investigators concluded that conventional techniques using H&E underestimate fatty change because of failure to identify microsteatosis, which accounted for 25% of the overall high-grade steatosis in this study.

Radiological methods. Different digital imaging procedures have been used to detect and quantify the fat content of the liver in vivo. Fatty droplets within hepatocytes will scatter ultrasound beams in such a way to produce the phenomenon of bright echotexture, in which the liver is more echogenic than the adjacent kidney. Freese and Lyons44 showed a linear relationship between total lipid concentration and ultrasound backscatter coefficient. However, others found that ultrasound provides acceptable levels of qualitative sensitivity without providing reliable quantitative information.45 Also, studies have shown marked intercenter differences, as well as interoperator differences in a given center.46

Magnetic resonance imaging (MRI) and spectroscopy allow the detection of small amounts of fat components in hepatocytes, but are less widely available and require considerably longer examination times and higher costs than CT.47-48 Liver density assessed by CT accurately reflects the presence of steatosis, and techniques to convert Hounsfield units into real fat volume fractions have been developed.49-50 This provides reproducible noninvasive measures of global hepatic fat content, with no risk for intrahepatic sampling error because of a lack of homogeneity in fat distribution.51 However, although a positive finding on CT or MRI has correlated positively with steatosis on biopsy, the converse recently was shown not to be the case, with 30% of negative scans by MRI and 24% of negative scans by CT associated with greater than 10% steatosis.52 Moreover, it must be emphasised that although useful as experimental tools, logistic constraints of scanning a hemodynamically unstable brain-dead donor, as well as the financial burden incurred, minimize the usefulness of these techniques in the clinical situation.

Tissue lipid content assay. It is possible to measure total lipid content in freeze-dried liver homogenates by extraction in chloroform:methanol and enzymatic analysis of total cholesterol,53 triglyceride,54 and phospholipid.55 However, these techniques are time consuming and have been reported only in experimental settings.

Miscellaneous. Conventional liver function tests have been unable to differentiate between grafts that go on to have adequate function and those with shortened survival in both retrospective and prospective analyses.56-57 This has led to investigation of more objective dynamic parameters, such as determination of monoethylglycinexylidide (MEGX).58 The MEGX test is based on the formation rate of the principle lignocaine metabolite MEGX by the cytochrome P-450 system in an oxidative reaction and has been evaluated in a number of studies as a method of assessing potential donor livers. Oellerich et al59 showed that low clearance levels correlated well with poor survival outcome. However, others failed to show an association of MEGX with the presence of moderate or severe steatosis.58

Laser Doppler flowmetry (LDF) is a noninvasive technique that allows continuous evaluation of microvascular perfusion.60 Its use for the measurement of flow in hepatic microcirculation has been validated in animal models, and LDF correlates well with invasive methods of measuring surface hepatic microcirculation.61 Seifalian et al26 showed a significant association between degree of steatosis and hepatic parenchymal microcirculation measured by LDF and suggested that
this may provide a useful tool to determine levels of steatosis. However, it would not provide useful data until the liver has been reperfused within the donor, at which point it would be impossible to discard unsatisfactory organs.

Hayashi et al.19 suggested measuring total liver blood flow at harvesting, graft weight change during preservation, and liver enzyme levels in the effluent during flushing. There is no evidence in the literature that any of these parameters were assessed and correlated with steatosis.

Potential for Novel Preservation Methods and Amelioration of Injury to Fatty Livers

Hepatocyte growth factor has ameliorated preservation injury in fatty livers, possibly by providing some protection to hepatocytes and sinusoidal lining cells.62 It also has ameliorated liver injury by carbon tetrachloride poisoning and lipopolysaccharide.63 Prostaglandin E1, an agent known to improve hepatic microcirculation, also has shown a beneficial effect in diminishing ischemic injury in a Zucker fatty transplant model.64 Beneficial effects of prostaglandin E1 include vasodilatation, inhibition of platelet aggregation and neutrophil activation, improved erythrocyte deformability, stabilization of lysosomal membranes, and stimulation of the regenerative capability of the liver.

Isolated perfusion has been investigated by many groups to allow the assessment of liver viability as a means of replicating the organ’s natural environment.65,66 The use of isolated perfusion to predict survival of steatotic livers before transplantation would merely require establishing parameters that correlate with survival. These may include hemodynamic parameters,67 as well as markers of synthetic function68 and liver injury69 assessed by sampling perfusate. If a supply of oxygen and nutrients is provided within a suitable perfusate, then continual normothermic preservation may become a viable reality, thus abrogating the increased sensitivity of fatty livers to cold preservation-reperfusion injury.

Future Directions

In view of the importance of fatty change in liver transplantation, it is surprising that fundamental issues regarding donor organ quality are still so poorly defined. Most surgeons would agree that much practiced clinically is based on individual preference and anecdotal evidence. Several key questions that might be addressed include: (1) Is histological assessment of a single biopsy specimen an accurate method of assessing steatosis? (2) Is there a clear-cut threshold of steatosis above which the risk for PNF outweighs the benefits of transplantation? (3) Should other modalities for the assessment of fatty change, such as CT, be used, or are logistic constraints insuperable? (4) Should normothermic perfusion be used as a means of both preservation and assessment of marginal livers?

Conclusions

Cadaveric brain-dead donors have a greater prevalence of steatosis than the rest of the healthy population, although the mechanism of induction remains unclear. The subjective ability of a surgeon to assess steatosis from macroscopic appearance and texture has been shown in several prospective series to be inaccurate and lead to transplantation of poor-quality organs. Assessment of steatosis is performed in some centers by donor and/or backbench biopsy of suspected fatty livers or on a uniform protocol basis. The latter has been shown in a single trial to significantly reduce the PNF rate.

Basing the decision of whether to use an organ on a single donor biopsy is only a partial solution to the assessment of steatotic livers. Fatty change has been shown to occur in a nonuniform fashion, creating the possibility for underestimation or overestimation from one biopsy. Also, no consensus agreement regarding which staining technique is most accurate has been reached. CT provides the best method of assessing global fatty change, although this would create a logistic and financial burden to the procurement process if adopted on a widespread scale. Thus, its use is severely limited. Machine perfusion may provide a method of viability assessment of marginal organs, although this has yet to be proved in an experimental model of steatosis.

High levels of macrovesicular steatosis (>60% on a single H&E-stained biopsy specimen) have a strong correlation with PNF. This deleterious effect of steatosis may result from increased sinusoidal resistance secondary to the physical interference of ballooned hepatocytes or direct injury to hepatocytes and/or sinusoidal lining cells after a period of cold preservation and reperfusion. Grafts showing this level of steatosis pose too great a risk for PNF and should not be considered for transplantation. Grafts with moderate degrees of steatosis (30% to 60%) should be considered in conjunction with other donor and recipient variables before a decision about their fate is made. Conversely, microvesicular steatosis does not appear to result in increased sensitivity of a liver to ischemia and/or reperfusion.
References


