

Defatting strategies in the current era of liver steatosis

Laura Ioana Mazilescu,^{1,2,3} Markus Selzner,¹ Nazia Selzner^{1,*}



Summary

Liver steatosis is emerging as a major cause of chronic liver disease worldwide, mainly due to the increasing rate of obesity, type 2 diabetes, and metabolic syndrome. Because of the increased incidence of liver steatosis, many organs are currently declined for transplantation despite high demand and waiting list mortality. Defatting strategies have recently emerged as a means of rapidly reducing liver steatosis to expand the pool of available organs. This review summarises advances in defatting strategies in experimental and human models of liver steatosis over the last 20 years.

© 2021 Published by Elsevier B.V. on behalf of European Association for the Study of the Liver (EASL). This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Liver transplantation represents the only life-saving therapeutic option for patients with acute liver failure, end-stage liver disease, and advanced hepatocellular carcinoma. Due to the overall excellent outcomes associated with liver transplantation, demand for liver grafts, which far exceeds organ availability, has continued to grow. As a result, both waiting times for patients listed for liver transplantation and waiting list mortality are increasing. Currently, many organs, including grafts from older donors, donors after cardiac arrest and fatty livers, are not used. Strategies to improve the quality of these grafts can potentially enable expansion of the current pool of available liver allografts for transplantation.

According to the World Health Organization, in 2016, 13% of the adults aged 18 and over were obese (BMI >30).¹ With increasing rates of obesity worldwide, the pool of potential donors is populated with fatty livers. Donor steatosis is a major risk factor for liver transplantation, as steatotic grafts are more susceptible to cold ischaemic injury, resulting in an increased risk of post-operative graft dysfunction, graft loss and requirement for re-transplantation.² Reducing the fat content in steatotic grafts, also called defatting, could allow access to this large donor pool, and potentially reduce the current organ shortage. Accordingly, there is much interest in developing defatting strategies to rapidly reduce graft steatosis prior to transplantation and to improve outcomes of liver transplantation with steatotic grafts.

Hepatic steatosis results from the accumulation of triacylglycerol in small or large lipid droplets (LDs) in the cytoplasm of hepatocytes due to abnormal fatty acid metabolism.^{3,4} Small LD accumulation does not displace the hepatocyte nuclei, which appears as microsteatosis on liver biopsy.

Microsteatotic livers can be transplanted with favourable results.⁵ In contrast, macrovesicular steatosis is caused by large LDs that cause displacement of the cell nucleus. Donor livers with severe macrovesicular steatosis have been reported to significantly increase the risk of primary and early graft dysfunction after liver transplantation.^{3,6,7} Lipid accumulation in hepatocytes is associated with endoplasmic reticulum, oxidative and mitochondrial stress and impaired autophagy, which can lead to cell damage and death.⁸ More than 20 years ago, Day and James proposed the 2 hit hypothesis to explain the pathogenesis of non-alcoholic fatty liver disease (NAFLD).⁹ According to this hypothesis, lipid accumulation in the form of triglycerides (TGs) is the first step in the development of NAFLD and represents the “first hit”. Lipid peroxidation due to oxidative stress in the context of lipid-loaded hepatocytes induces inflammation and necrosis, thus representing the “second hit”.⁹ In contrast, some other authors believe that alone TG accumulation in the form of LDs does not result in cellular injury and inflammation,^{10,11} an alternative also recognised by Day and James.¹² The accumulation of TGs occurs in parallel with the generation of fatty acid metabolites, which have been associated with lipotoxic hepatocellular injury, resulting in endoplasmic reticulum stress, inflammation, apoptosis, and necrosis.^{13,14}

NAFLD includes the non-alcoholic fatty liver (NAFL) form, which histologically is characterised by steatosis with no or minor inflammation, and non-alcoholic steatohepatitis (NASH), which histologically is characterised by inflammation with or without fibrosis.¹⁵ About 40% of patients with NAFLD develop fibrosis, the progression of which is usually faster in patients with NASH.¹⁶ Approximately 5–18% of the patients with NASH develop cirrhosis, and about 38% of patients with NASH and

Keywords: Macrosteatosis; ischemia-reperfusion injury; machine perfusion; liver transplantation

Received 22 November 2020; received in revised form 14 February 2021; accepted 19 February 2021; available online 13 March 2021

¹Ajmera Transplant Program, Toronto General Hospital, Ontario, Canada; ²Division of Nephrology, The Hospital for Sick Children, Toronto, Ontario, Canada; ³Department of General, Visceral, and Transplantation Surgery, University Hospital Essen, Essen, Germany

* Corresponding author. Address: Multi-Organ Transplant Program, Toronto General Hospital, 585 University Avenue, 11 PMB-178 Toronto, ON, Canada M5G 2N2. E-mail address: nazia.selzner@uhn.ca (N. Selzner).



fibrosis develop cirrhosis. Patients with NASH and either fibrosis or cirrhosis are at an increased risk of hepatocellular carcinoma.

The endoplasmic reticulum, oxidative and mitochondrial stress caused by lipid accumulation exacerbate the fragility of the liver when exposed to ischaemia-reperfusion injury, which could explain why the transplantation of steatotic livers results in worse outcomes. The mechanisms of preservation-related injury of fatty grafts are amplified by exposing the liver to a cold anoxic environment. Strategies to improve the outcome of liver transplantation with steatotic grafts include minimising cold anoxic storage by using normothermic perfused preservation or defatting the grafts during the preservation time. Both techniques are often combined in a synchronous approach since defatting protocols usually require an active metabolism. New evidence has shown that defatting of steatotic donor livers has the potential to improve organ quality, therefore allowing us to increase the usage of such organs. We acknowledge that defatting cannot be performed in any steatotic liver; **there is likely a point beyond which liver injury due to fat deposition cannot be reversed; the precise 'point-of-no-return', however, remains to be determined.**

Key point

Steatosis is a frequent reason for potential liver grafts to be declined.

We will review the pharmacological agents used for defatting as well as *in vitro* and *in vivo* defatting strategies, including recent progress with the advent of machine perfusion.

Pharmacological defatting

Several drugs have been reported to reduce hepatic fat content by increasing fat utilisation.¹⁷ Table 1 and Fig. 1 summarise agents that have been used for defatting and their mechanisms of action in cell culture and animal models.

Lipophilic ligand-activated transcription factors, such as peroxisome proliferator-activated receptors (PPARs), have been shown to influence lipid and glucose metabolism, resulting in an increase in high-density lipoproteins.^{18,30–32} Forskolin has been found to increase intracellular cyclic AMP, which in turn enhances β -oxidation and ketogenesis.¹⁹ L-carnitine is one of the substrates necessary for the oxidation of long-chain fatty acids.²⁰ It has also been demonstrated to influence glucose metabolism, by inducing genes that participate in glycolysis and suppress

neoglucogenesis.^{21,22} Scoparone, an androstane receptor ligand, has been shown to improve the viability of Sprague-Dawley rat hepatocytes preserved in Euro-Collins solution.²⁴ It also has vasodilator properties, enhancing the production of prostacyclin and resulting in platelet aggregation.³³ Scoparone, along with a combination of chlorogenic acid, rhein, geniposide and emodin, are the main components of a traditional Chinese medicine formula, which has been described to enhance adiponectin and upregulate PPAR- γ , resulting in reversal of hepatocyte steatosis.²³ Visfatin, was initially described as an insulin-mimetic adipokine, which in combination with other drugs could reduce intracellular lipid content by promoting export from cultured hepatocytes.²⁵ Rapamycin, an inhibitor of the mammalian target of rapamycin, has been proven to enhance exogenous fatty acid oxidation,²⁶ promote lipogenesis,²⁷ induction of TG secretion and macroautophagy.²⁸ Necrosulfonamide is an inhibitor of the mixed lineage kinase domain like pseudokinase, which has been reported to regulate insulin sensitivity and TG deposition in the liver.²⁹ Different combinations of these drugs have been studied in cell culture and animal models to determine their potential as defatting agents.

In vitro defatting strategies

Several studies have investigated the potential of different defatting agents *in vitro* (Table 2). Using a model of rat hepatocytes, Nativ *et al.* investigated the effects of accelerated macrosteatosis reduction on hepatocyte viability.²² Steatosis was induced by exposing rat hepatocytes to a fatty acid-supplemented media for 6 days. Subsequently, cell media was replaced by either regular media or media with steatosis reduction supplements (SRS) containing a cocktail of forskolin (a glucagon mimetic), the PPAR α agonist GW7647, scoparone, the PPAR δ agonist GW501516, hypericin (a pregnane X receptor [PXR] ligand), visfatin (an adipokine) and amino acids. Hepatocytes cultured in media supplemented with SRS exhibited a 4-fold faster reduction of macrosteatosis compared to those cultured in regular media, with comparable viability in both cases. Moreover, hepatocytes exposed to SRS showed a quicker recovery of urea secretion and bile canalicular function, indicating that the functional recovery of hepatocytes is either dependent on the macrosteatosis reduction time or the direct influence of the SRS. In a follow-up study with the same model, Nativ *et al.* investigated the potency of defatting agents in alleviating hypoxia/reoxygenation injury.³⁴ Cells were cultured

Table 1. Defatting agents.

Drug	Mechanism of action	Observed effects
Peroxisome proliferator-activated receptors (PPAR)	Influences lipoprotein metabolism by regulating the expression of several genes responsible for the synthesis, remodeling and removal of plasma lipids and lipoproteins ¹⁸	Decrease in plasma TG levels Increase in high-density lipoproteins
Forskolin	Increases intracellular cyclic AMP, which in turn enhances β -oxidation and ketogenesis ¹⁹	Increased cellular oxidation of fatty acids ¹⁷
L-carnitine	Involved in the oxidation of long-chain fatty acids, glycolysis, gluconeogenesis ^{20,21}	Improved hepatocyte resistance to hypoxia/reoxygenation injury ²²
Scoparone	PPAR- γ upregulation ²³	Improved hepatocytes viability ²⁴
Visfatin	Exhibits insulin-mimetic effects ²⁵	Induces TG accumulation in preadipocytes ²⁵
Rapamycin (mTOR)	Promotes fatty acid oxidation, ²⁶ lipogenesis ²⁷ and TG secretion and macroautophagy ²⁸	NR
Necrosulfonamide	Controls insulin sensitivity and TG depositions in the liver ²⁹	Enhanced fat depletion in PHH, increased efficiency of other defatting agents ²⁰

NR, not reported; PHHs, primary human hepatocytes; PPAR, peroxisome proliferator-activated receptors; TG, triglyceride.

either in fresh hepatocyte media supplemented with SRS or supplemented with SRS and L-carnitine and exposed to a gas mixture of either 90%air/10%CO₂ or 90%O₂/10%CO₂. L-carnitine is implicated in transferring free fatty acids from the cytosol to the mitochondria, so the authors hypothesised that L-carnitine would also favour β-oxidation and decrease TG storage. They reported that cells cultured in SCS and L-carnitine and exposed to hyperoxia showed a 60% reduction in TG and a 70% increase in

β-oxidation. Also, the same treatment significantly increased ATP levels and lowered lactate dehydrogenase release. Together these experiments suggest that the SRS cocktail might improve cell function through other mechanisms, such as improved mitochondrial β oxidation and increased ATP content.

Yarmush *et al.* induced hepatocyte steatosis by exposing human hepatoma cells to free fatty acids.³⁵ Defatting was attempted using the same cocktail used by Nativ *et al.*^{22,34} Exposure to

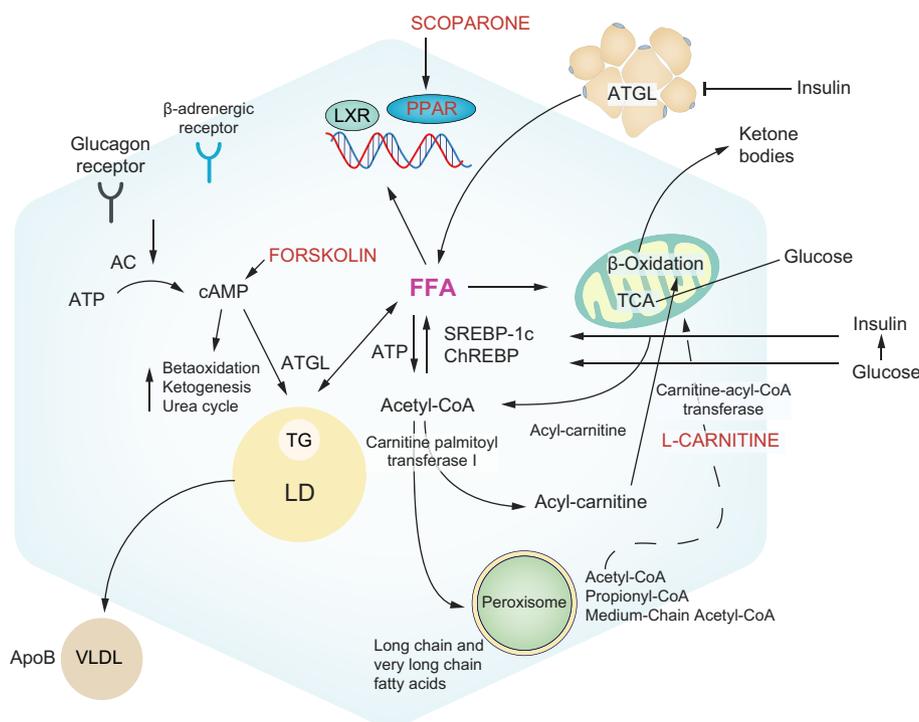


Fig. 1. Potential mechanisms of action of different agents used for defatting. Forskolin increases intracellular cAMP, which in turn enhances β-oxidation and ketogenesis. Fatty acids can act as ligands to nuclear receptors, such as LXR or PPAR; PPARs influence lipid and glucose metabolism. Peroxisomes can oxidize fatty acids; the different acyl-CoA esters produced in the peroxisomes are transferred to the mitochondria for complete oxidation. Scoparone has been described to enhance adiponectin and upregulate PPAR-γ, resulting in reversal of hepatocyte steatosis. AC, adenylate cyclase; ApoB, apolipoprotein B; ATGL, adipose triglyceride lipase; cAMP, cyclic AMP; ChREBP, carbohydrate response element binding protein; FFA, free fatty acids; LD, lipid droplets; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptors; SREBP-1c, sterol regulatory element binding protein-1c; TCA, tricarboxylic acid cycle; TG, triglycerides; VLDL, very low-density lipoproteins.

Table 2. In vitro studies using pharmacological agents for defatting.

First author	Year	In vitro model	Defatting strategy	Effect of DA
Nativ <i>et al.</i> ²²	2013	Rat hepatocytes	Cell media ± forskolin, PPAR α and δ agonist, scoparone, hypericin, visfatin, amino acids	Faster steatosis reduction; quicker recovery of urea secretion and bile canalicular function
Nativ <i>et al.</i> ³⁴	2014	Rat hepatocytes	Cell media + forskolin, PPAR α and δ agonist, scoparone, hypericin, visfatin, amino acids ± L-carnitine ± 90% O ₂	Presence of L-carnitine and hyperoxia - higher reduction in TG, increase in β-oxidation and ATP levels
Yarmush <i>et al.</i> ³⁵	2016	Human hepatoma cells	Cell media ± forskolin, PPAR α and δ agonist, scoparone, hypericin, visfatin amino acids ± 90% O ₂	Accelerated defatting, especially in hyperoxia; increase in β-oxidation, tricarboxylic acid and urea cycle
Boteon <i>et al.</i> ³⁶	2018	PHH HIEC Human cholangiocytes	Cell media ± forskolin, PPAR α and δ ligand, hypericin, scoparone, visfatin, L-carnitine	PHH - decrease in LD and TG content; increase in the viability of PHH and cholangiocytes; no cytotoxic effect on HIEC
Aoudjehane <i>et al.</i> ²⁰	2020	PHH Primary hepatocytes from human fatty liver Human precision cut liver slices	Forskolin, L-carnitine, PPARα agonist, rapamycin, necrosulfonamide	Decrease in LD and TG content and in endoplasmic reticulum stress and production of reactive oxygen species

DA, defatting agents; HIEC, hepatic endothelial cells; LD, lipid droplets; PHHs, primary human hepatocytes; PPAR, peroxisome proliferator-activated receptors; TG, triglyceride

basal media without any defatting cocktail resulted in a 50% reduction in TG content within 24 h both under normoxic (21% O₂) and hyperoxic (90% O₂) conditions. Extending the defatting period to 48 h did not result in a further decrease in TG content. Using the defatting cocktail accelerated the defatting process with an 83% decrease in TG content after 48 h under hyperoxia. Similar to the previous study, defatting resulted in an increase in β -oxidation, as well as tricarboxylic acid and urea cycle activity.

Boteon and colleagues also investigated the potential of a defatting drug cocktail to reduce the *in vitro* lipid content of primary human hepatocytes (PHHs).³⁶ The authors used a cocktail of forskolin, PPAR α ligand, hypericin, scoparone, PPAR δ ligand, visfatin and L-carnitine that were previously tested both in rat cells and human hepatoma cells. Hypericin has been demonstrated to activate xenobiotic pathways, however, its involvement in lipid metabolism remains unclear.²⁵ Steatosis was induced in PHHs using a combination of saturated and unsaturated free fatty acids. Cells were subsequently treated for 48 h with the defatting cocktail. In order to assess the effects of this cocktail on non-parenchymal liver cell viability, the same treatment was applied to human intrahepatic endothelial cells (HIECs) and human cholangiocytes. After 48 h of incubation with the drugs, fat loaded PHHs showed a 54% decrease in the total area of cytoplasmic LD, a decrease in intracellular TG content and a shift from macrovesicular steatosis to microvesicular steatosis. Moreover, the defatting cocktail increased the viability of PHHs by 11% and cholangiocytes by 25%, while exhibiting no cytotoxic effects on HIECs.

Key point

Promising results have been achieved with pharmacological defatting strategies; however, none have so far been approved for usage in humans.

A recent study tested a novel defatting cocktail including previously reported forskolin, L-carnitine, PPAR α agonist, as well as 2 new mediator-targeting pathways involved in fat storage, rapamycin and necrosulfonamide.²⁰ PHHs with free fatty acid-induced steatosis were treated with the cocktail for 24 h. Hepatocytes treated with the defatting cocktail showed a 29% decrease in intracellular LDs and a 30% decrease in TG content. A similar effect was observed in primary hepatocytes from human fatty liver samples with a 36% decrease in LDs and a 29% decrease in TG content in the treatment group. To further test the defatting potency of this cocktail, human precision cut liver slices were treated with the cocktail with or without necrosulfonamide. Again, lipid inclusions and TG content were significantly reduced by 38% and 50%, respectively. Moreover, in all 3 models, a decrease in endoplasmic reticulum stress and in the production of reactive oxygen species was observed.

Ex situ machine perfusion

The increased usage of extended criteria grafts and the detrimental effects of static cold storage on these grafts have triggered interest in *ex situ* machine perfusion as an alternative preservation technique to decrease preservation injury. Besides improvement of graft quality and survival, *ex situ* perfusion also provides the opportunity for assessment and treatment while preserving the organ. In this context, the use of defatting strategies during *ex situ* machine perfusion has emerged and been rapidly adopted in the last few years.

Preclinical studies

Hypothermic machine perfusion

Hypothermic machine perfusion has been reported to improve the preservation of steatotic livers compared to static cold storage.³⁷ Bessems *et al.* used a choline-methionine-deficit diet to induce liver steatosis in Wistar rats. Rat livers were perfused for 24 h at 4°C or maintained on ice in the control group. The authors showed that machine perfused grafts had higher perfusate flow, increased hyaluronic acid and ammonia clearance as well as higher urea production compared to cold stored grafts. Furthermore, bile production, oxygen consumption and ATP levels were higher in the study group, indicating a healthier graft quality in the perfused group. Similar results were found by Kron and colleagues using a similar model of machine perfused fatty livers followed by transplantation of the perfused grafts.³⁸ After inducing liver macrosteatosis of $\geq 60\%$ in rats using a choline-methionine-deficit diet, grafts were retrieved and transplanted after either minimal static cold storage (SCS), 12 h of SCS or 12 h of SCS followed by 1 h of oxygenated hypothermic machine perfusion (HOPE). A short period of HOPE treatment before transplantation resulted in decreased reperfusion injury, as indicated by less oxidative stress, nuclear damage, Kupffer and endothelial cell activation. However, steatosis content was not influenced by HOPE. In the same study, the authors also reported results from 6 moderately steatotic human livers which received HOPE treatment before implantation. Outcomes after transplantation were consistent with animal data.

Subnormothermic and normothermic machine perfusion

Normothermic *ex situ* machine perfusion (NEsLP) alone without use of any defatting cocktail has been shown to reduce hepatocyte steatosis. In a porcine model of diet-induced fatty liver, a decrease of liver steatosis from 28% to 15% was observed after 48 h of NEsLP perfusion.³⁹ However, the addition of defatting cocktails appears to enhance the effect of machine perfusion.

Using a rat model of steatotic liver, Nagrath *et al.* reported a 65% reduction in liver TG content after a 3 h period of normothermic perfusion with a defatting cocktail.²⁵ The defatting cocktail included a PPAR α ligand (GW7647), a PPAR δ ligand (GW501516), hypericin, scoparone, forskolin and visfatin. When control livers were perfused for 3 h without the defatting agents, a 30% reduction in TG content was observed, again demonstrating the potential defatting effect of machine perfusion alone. The authors concluded that the decrease in fat droplets was related to increased β -oxidation due to better oxygen availability.

The same group investigated the effectiveness of subnormothermic (20°C) machine perfusion in reducing fat content in steatotic livers.⁴⁰ Livers from obese Zucker rats were perfused at room temperature with a perfusate supplemented with (defatting group) or without (control group) the same defatting agents used in the previous study.³⁹ However, no significant reduction in lipid content was observed in both groups after 6 h of perfusion, indicating the possible importance of active metabolism for successful defatting.

Key point

Ex situ machine perfusion has the potential to contribute to defatting strategies, however no exact treatment for steatosis has been established.

Glial cell-line derived neurotrophic factor (GDNF) has been reported as another alternative strategy to reduce liver steatosis during *ex vivo* perfusion.⁴¹ In an initial study, Vakili *et al.* had determined that GDNF has a protective effect against high-fat diet (HFD)-induced hepatic steatosis, by reducing PPAR γ expression.⁴² Steatotic and lean livers were perfused with a perfusate containing either the vehicle (negative control), GDNF, or defatting cocktail (same defatting cocktail described by Nagrath *et al.*³⁹). Perfusion with GDNF resulted in a significant reduction (>40%) in fat content in HFD-fed mice, compared to non-perfused livers from HFD-fed mice and HFD-fed mice perfused with vehicle. Perfusion with GDNF also resulted in a significantly higher reduction in TG content. Moreover, perfusion with GDNF proved more effective in decreasing TG content than perfusion with the defatting cocktail.

In a recent study, Raigani *et al.* subjected steatotic rat livers to NEsLP with a defatting drug cocktail.⁴³ The defatting cocktail was based on drugs that were proven to be effective in cell culture models: forskolin, scoparone, hypericin, visfatin, L-carnitine, and amino acids. After 6 h of perfusion, the defatted livers showed increased viability, demonstrated by lower perfusate lactate and better bile quality (lower bile lactate and higher bile bicarbonate). Moreover, the authors reported that defatted livers showed decreased expression of inflammatory mediators, such as NF- κ B and tumour necrosis factor- α , and of apoptotic markers caspase 3 and Fas cell surface death receptor. Gene expression of the mitochondrial fatty acid oxidation markers acyl-CoA oxidase 1 and carnitine palmitoyltransferase 2 were upregulated in the treated grafts. However, no significant reduction in macrosteatosis was achieved by defatting perfusion. The authors concluded that pharmaceutical lipid modulation in steatotic rat livers has the potential to improve liver function and that a significant decrease in macrosteatosis might not be the most important goal of perfusion.

Clinical trials

Hypothermic machine perfusion for liver preservation has recently been translated into human trials with promising first results.⁴⁴ In 1 study, discarded human livers (mainly because of macrosteatosis) and potentially transplantable livers that could not be allocated were preserved for 24 h with hypothermic machine perfusion without oxygen.⁴⁵ Steatotic livers released more hepatocellular injury markers (aspartate aminotransferase and lactate dehydrogenase) and had a lower ATP recovery rate than the potentially transplantable livers. Steatotic livers tended to show more irreversibly damaged mitochondria at the end of perfusion compared to the potentially transplantable livers.

Normothermic *ex situ* machine perfusion of liver grafts has increasingly been used in the recent years with positive outcomes.^{46–48} More recently, several groups have assessed NEsLP for defatting human grafts. In a series of 10 liver grafts, the Cleveland group investigated whether NEsLP alone results in a reduction of liver steatosis in human grafts.⁴⁹ After 24 h of perfusion, tissue steatosis did not decrease. There was an increase in the TG content of the perfusate from the beginning to the end of perfusion. Perfusate total cholesterol, high-density lipoproteins and low-density lipoproteins decreased over the course of perfusion. The authors speculate that their findings are not in accordance with the animal observations because of the differences between species and the way steatosis is induced in the animal models (*vs.* the chronic fat accumulation in human livers). However, the authors argued that the increased TG levels in the perfusate indicate active metabolism of the grafts, which suggest that NEsLP could be a promising platform alongside defatting cocktails.

More recently, Boteon and colleagues investigated the capacity of a combination of drugs to increase the metabolism of intracellular lipids.⁵⁰ Five discarded human livers were subjected to NEsLP using a blood and human albumin-based perfusate, which was supplemented with a cocktail of defatting agents: forskolin, hypericin, scoparone, visfatin and L-carnitine (treatment group). As a control group, 5 more grafts were perfused under the same condition, without adding the cocktail of defatting agents. Grafts in the treatment group showed increased ketogenesis, solubility of the TG and ATP synthesis compared with the control group. There was also a significantly greater reduction in tissue TG levels (which declined by 38%) and macrovesicular steatosis (decreased by 50% after 12 h) in the treatment group. During the 12 h of perfusion, grafts in the treatment group showed higher lactate clearance and bile production, both markers of graft viability. Despite these promising results, it is important to consider that safety data is not available for some of the drugs used in the defatting cocktail, for example hypericin and PPAR, and their toxicity in humans has not been investigated.⁵¹

Living liver donation

Living donor liver transplantation is accepted as a safe strategy to increase the pool of liver organs for transplantation. Potential live donors with evidence of liver steatosis >10% are excluded from donation in many transplant centres worldwide and are usually referred to a dietician and have to follow dietary restrictions and an exercise regimen for a prolonged time.

Table 3. Defatting strategies in living liver donors.

First author	Year	Number of treated donors	Number of transplantations performed	Defatting strategy	Duration of treatment	Outcomes
Nakamuta <i>et al.</i> ⁵²	2005	11	7	Protein-rich diet, exercise and bezafibrate	2–8 weeks	Graft function of defatted livers similar to that of lean grafts
Clavien <i>et al.</i> ⁵³	2010	3	3	Omega-3 fatty acids	4 weeks	NR
Doyle <i>et al.</i> ⁵⁴	2016	16	14	Optifast	4 weeks	Graft function of defatted livers similar to that of lean grafts
Chung <i>et al.</i> ⁵⁵	2020	7	7	NR, weight reduction was aimed	8–16 weeks	Graft function of defatted livers similar to that of lean grafts
Fujii <i>et al.</i> ⁵⁶	2020	8	8	Diet, exercise \pm statins	5–16 weeks	Graft function of defatted livers similar to that of lean grafts

NR, not reported.

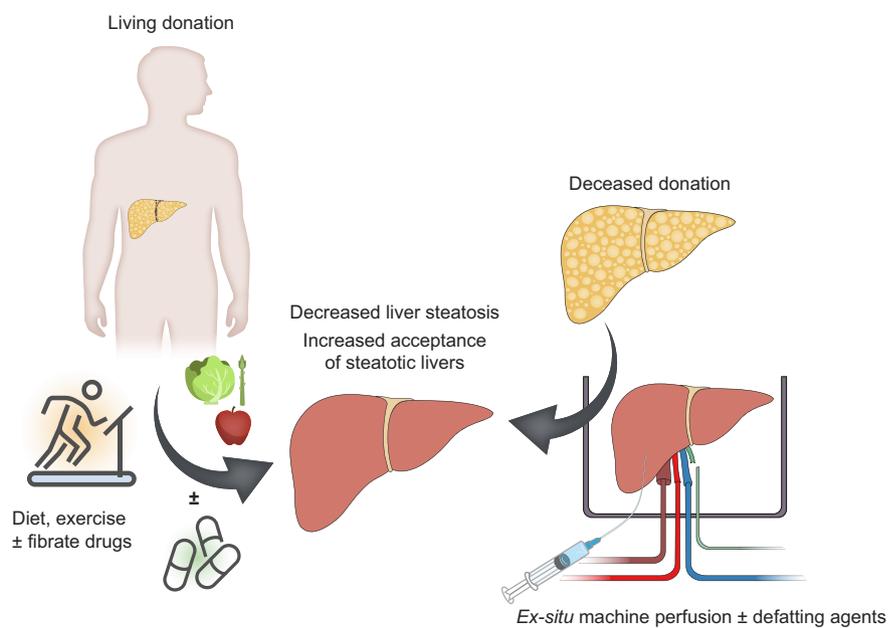


Fig. 2. Possible strategies to reduce liver steatosis in human livers.

In the current era of obesity, strategies to reduce fatty liver in potential donors are crucial (Table 3). An earlier study from a Japanese group investigated if the combination of a protein-rich diet, exercise and bezafibrate for 2–8 weeks can reduce the risk of liver injury in living-donor liver transplantation candidates with steatosis.⁵² They found that this regimen significantly reduced liver steatosis, determined by liver biopsy, from about 30% before treatment to around 12% after treatment. Body weight and BMI were also significantly reduced. Eleven possible donors were treated, and among them 7 proceeded to live donation. Graft function and outcomes post-transplantation were similar in the recipients of these grafts compared to living donor recipients who received a lean graft.

Clavien *et al.* also reported a successful reduction (>30%) of liver steatosis in 3 living donors using omega-3 fatty acids.⁵³ The reduction was achieved within 1 month of treatment and all 3 donors proceeded to live donation. Details regarding graft function and outcomes post-transplantation were not reported.

We reported our experience with Optifast, a very low-calorie diet meal replacement, in reducing hepatic steatosis in candidates for living donor liver transplantation.⁵⁴ Sixteen potential live donors followed an Optifast-based diet for at least 4 weeks. No exercise regimen was required. A short course of Optifast was well tolerated and resulted in a consistent and rapid reduction of hepatic steatosis in all donors. All post-Optifast biopsies showed <10% macrovesicular steatosis after the completion of treatment. Optifast-treated donors who proceeded to hepatectomy experienced similar postoperative outcomes as non-Optifast-treated donors. Transplant recipients also had equivalent outcomes after receiving grafts from either Optifast-treated donors or control donors without steatosis. There were no significant differences in surgical complications, retransplantation rates, or mortality.

Key point

Diet and exercise before transplantation have proven effective and should be further explored as a defatting strategy.

More recently, Chung and colleagues investigated the efficacy of weight reduction in living liver donors with >30% macrovesicular steatosis and BMI >25 kg/m².⁵⁵ The control group included donors with <30% macrovesicular steatosis. In the weight reduction group, a significant BMI and steatosis reduction was achieved before donation. Weight reduction was achieved over a period of 8 to 16 weeks and had to be maintained for at least 8 weeks prior to surgery. Post-transplantation outcomes of both donors and recipients were comparable to the control group. Overall, the study demonstrated that steatosis reduction is feasible albeit over several weeks, which in some instances may not be possible due to the recipient's health. Similar results were also reported by Fujii and colleagues.⁵⁶ In this study, potential donors diagnosed with fatty liver via CT scan were prescribed a restricted calorie intake <1,600 kcal/day diet, exercise at least 3 times a week for >20 min and statins if hyperlipidaemia was present. The weight reduction period was 35 to 109 days. The control group included patients with no signs of fatty liver via imaging. After transplantation, there were no significant differences between the treatment group and the control group in terms of patient and graft survival, early graft function and ascites volume.

Bariatric surgery has been described in the context of liver defatting. One group reported the case of an obese patient who donated a liver lobe for transplantation after undergoing sleeve gastrectomy with positive results.⁵⁷ Bariatric surgery has so far not been widely used as a defatting strategy; however, it might represent a possible option in the future.

Discussion

Liver steatosis results in cellular changes which increase ischaemia-reperfusion injury and result in worse outcomes after liver transplantation; therefore, fatty livers are not usually used for transplantation.¹⁷ Defatting strategies represent an important opportunity to increase the pool of liver grafts (Fig. 2). Several strategies have been investigated to date, including the use of pharmacological agents, which can potentially be applied during NELS for steatotic grafts, and diet for living liver donors (Fig. 2).

The potential defatting effects of several pharmaceutical agents have been proven in different cell culture models. These agents target lipid and glucose metabolism,^{30–32} the oxidation of long-chain fatty acids²⁰ and mitochondrial β -oxidation and ketogenesis.¹⁹ To date, only 1 study has successfully demonstrated the defatting potential of these cocktails in declined human grafts.⁵⁰ Despite promising results, several of the drugs used in the defatting cocktails are currently not approved for usage in a clinical setting because safety studies are still missing.

In the setting of living liver donation, efforts have been targeted toward fat reduction using diet and exercise.^{54–56} Studies in these setting have shown that weight reduction before donation is safe and grafts transplanted from these donors have comparable outcomes to grafts from donors without signs of

steatosis. However, to this point, these studies have included a very limited number of patients. Future studies need to confirm these findings in larger cohorts of patients. In addition, the length of these strategies is often extensive and sometimes unrealistic due to recipients' health. Novel preservation techniques, such as normothermic machine perfusion, enable defatting strategies to be applied prior to transplantation, without (or only minimally) exposing donors or recipients to potential toxicity. These techniques could potentially be expanded in the setting of living liver donation.

Conclusions

Steatotic liver grafts represent an increasing proportion of the donor pool. Due to the inferior outcomes after liver transplantation, many centres refuse to use these grafts. Defatting strategies are a promising solution to this problem, but only a limited number of studies have investigated this approach. NELS represents an excellent opportunity for organ assessment and treatment and could be used as a defatting platform if defatting agents become available. Defatting through diet and exercise has been successfully reported for living liver donors. However, reports are very limited and further studies are required to validate this method.

Abbreviations

GDNF, glial cell-line derived neurotrophic factor; HFD, high-fat diet; HIEC, hepatic endothelial cells; HOPE, hypothermic machine perfusion; LDs, lipid droplets; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NELS, normothermic *ex situ* machine perfusion; PHHs, primary human hepatocytes; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; SCS, static cold storage; SRS, steatosis reduction supplements; TG, triglyceride.

Financial support

This work has been supported by grants from the Deutsche Forschungsgemeinschaft (MA 8516/1-1 to L.I.M.).

Conflict of interest

The authors declare no conflict of interest related to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All three authors contributed equally to the production of this manuscript.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhepr.2021.100265>.

References

- <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.
- Goldaracena N, Barbas AS, Selzner M. Normothermic and subnormothermic *ex-vivo* liver perfusion in liver transplantation. *Curr Opin Organ Transpl* 2016;21(3):315–321.
- Spitzer AL, Lao OB, Dick AA, Bakthavatsalam R, Halldorson JB, Yeh MM, et al. The biopsied donor liver: incorporating macrosteatosis into high-risk donor assessment. *Liver Transpl* 2010;16(7):874–884.
- Parekh S, Anania FA. Abnormal lipid and glucose metabolism in obesity: implications for nonalcoholic fatty liver disease. *Gastroenterology* 2007;132(6):2191–2207.
- Nativ NI, Maguire TJ, Yarmush G, Brasaemle DL, Henry SD, Guarrera JV, et al. Liver defatting: an alternative approach to enable steatotic liver transplantation. *Am J Transpl* 2012;12(12):3176–3183.
- Perez-Daga JA, Santoyo J, Suárez MA, Fernández-Aguilar JA, Ramírez C, Rodríguez-Cañete A, et al. Influence of degree of hepatic steatosis on graft function and postoperative complications of liver transplantation. *Transpl Proc* 2006;38(8):2468–2470.
- de Graaf EL, Kench J, Dilworth P, Shackel NA, Strasser SI, Joseph D, 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(3):540–546.
- Machado MV, Cortez-Pinto H. Non-alcoholic fatty liver disease: what the clinician needs to know. *World J Gastroenterol* 2014;20(36):12956–12980.
- Day CP, James OFW. Steatohepatitis: a tale of two “hits”? *Gastroenterology* 1998;114(4):842–845.
- Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008;28(04):370–379.
- Choi SS, Diehl AM. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Curr Opin Lipidol* 2008;19(3):295–300.
- Day CP, James OF. Hepatic steatosis: innocent bystander or guilty party? *Hepatology* 1998;27(6):1463–1466.
- Anderson N, Borlak J. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacol Rev* 2008;60(3):311–357.
- Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology* 2010;52(2):774–788.
- Calzadilla Bertot L, Adams LA. The natural course of non-alcoholic fatty liver disease. *Int J Mol Sci* 2016;17(5):774.
- Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13(4): 643–e40.
- Boteon YL, Boteon A, Attard J, Mergental H, Mirza DF, Bhogal RH, et al. *Ex situ* machine perfusion as a tool to recondition steatotic donor livers: troublesome features of fatty livers and the role of defatting therapies. A systematic review. *Am J Transpl* 2018;18(10):2384–2399.
- Kersten S. Peroxisome proliferator activated receptors and obesity. *Eur J Pharmacol* 2002;440(2-3):223–234.
- Pégorier JP, Garcia-Garcia MV, Prip-Buus C, Duée PH, Kohl C, Girard J. Induction of ketogenesis and fatty acid oxidation by glucagon and cyclic AMP in cultured hepatocytes from rabbit fetuses. Evidence for a decreased

- sensitivity of carnitine palmitoyltransferase I to malonyl-CoA inhibition after glucagon or cyclic AMP treatment. *Biochem J* 1989;264(1):93–100.
- [20] Aoudjehane L, Gautheron J, Le Goff W, Goumard C, Gilazeau J, Nget CS, et al. Novel defatting strategies reduce lipid accumulation in primary human culture models of liver steatosis. *Dis Model Mech* 2020;13(4). dmm042663.
 - [21] Keller J, Ringseis R, Priebe S, Guthke R, Kluge H, Eder K. Effect of L-carnitine on the hepatic transcript profile in piglets as animal model. *Nutr Metab (Lond)* 2011;8:76.
 - [22] Nativ NI, Yarmush G, Chen A, Dong D, Henry SD, Guarrera JV, et al. Rat hepatocyte culture model of macrosteatosis: effect of macrosteatosis induction and reversal on viability and liver-specific function. *J Hepatol* 2013;59(6):1307–1314.
 - [23] Li JY, Cao HY, Sun L, Sun RF, Wu C, Bian YQ, et al. Therapeutic mechanism of Yin-Chén-Hào decoction in hepatic diseases. *World J Gastroenterol* 2017;23(7):1125–1138.
 - [24] Cho HR, Choi DH, Ko BK, Nam CW, Park KM, Lee YJ, et al. Cold preservation of rat cultured hepatocytes: the scoparone effect. *Transpl Proc* 2000;32(7):2325–2327.
 - [25] Nagrath D, Xu H, Tanimura Y, Zuo R, Berthiaume F, Avila M, et al. Metabolic preconditioning of donor organs: defatting fatty livers by normothermic perfusion ex vivo. *Metab Eng* 2009;11(4-5):274–283.
 - [26] Brown NF, Stefanovic-Racic M, Sipula IJ, Perdomo G. The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. *Metabolism* 2007;56(11):1500–1507.
 - [27] Chen H, Shen F, Sherban A, Nocon A, Li Y, Wang H, et al. DEP domain-containing mTOR-interacting protein suppresses lipogenesis and ameliorates hepatic steatosis and acute-on-chronic liver injury in alcoholic liver disease. *Hepatology* 2018;68(2):496–514.
 - [28] Zhou W, Ye S. Rapamycin improves insulin resistance and hepatic steatosis in type 2 diabetes rats through activation of autophagy. *Cell Biol Int* 2018;42(10):1282–1291.
 - [29] Xu H, Du X, Liu G, Huang S, Du W, Zou S, et al. The pseudokinase MLKL regulates hepatic insulin sensitivity independently of inflammation. *Mol Metab* 2019;23:14–23.
 - [30] Luquet S, Lopez-Soriano J, Holst D, Gaudel C, Jehl-Pietri C, Fredenrich A, et al. Roles of peroxisome proliferator-activated receptor delta (PPARdelta) in the control of fatty acid catabolism. A new target for the treatment of metabolic syndrome. *Biochimie* 2004;86(11):833–837.
 - [31] Nagasawa T, Inada Y, Nakano S, Tamura T, Takahashi T, Maruyama K, et al. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. *Eur J Pharmacol* 2006;536(1-2):182–191.
 - [32] Tamura K, Ono A, Miyagishima T, Nagao T, Urushidani T. Profiling of gene expression in rat liver and rat primary cultured hepatocytes treated with peroxisome proliferators. *J Toxicol Sci* 2006;31(5):471–490.
 - [33] Huang H-C, Lee C-R, Weng Y-I, Lee M-C, Lee Y-T. Vasodilator effect of scoparone (6,7-dimethoxycoumarin) from a Chinese herb. *Eur J Pharmacol* 1992;218(1):123–128.
 - [34] Nativ NI, Yarmush G, So A, Barminko J, Maguire TJ, Schloss R, et al. Elevated sensitivity of macrosteatotic hepatocytes to hypoxia/reoxygenation stress is reversed by a novel defatting protocol. *Liver Transplant* 2014;20(8):1000–1011.
 - [35] Yarmush G, Santos L, Yarmush J, Koundinyan S, Saleem M, Nativ NI, et al. Metabolic flux distribution during defatting of steatotic human hepatoma (HepG2) cells. *Metabolites* 2016;6(1):1.
 - [36] Boteon YL, Wallace L, Boteon A, Mirza DF, Mergental H, Bhogal RH, et al. An effective protocol for pharmacological defatting of primary human hepatocytes which is non-toxic to cholangiocytes or intrahepatic endothelial cells. *PLoS One* 2018;13(7):e0201419.
 - [37] Bessems M, Doorschodt BM, Kolkert JL, Vetelainen RL, van Vliet AK, Vreeling H, et al. Preservation of steatotic livers: a comparison between cold storage and machine perfusion preservation. *Liver Transpl* 2007;13(4):497–504.
 - [38] Kron P, Schlegel A, Mancina L, Clavien PA, Dutkowski P. Hypothermic oxygenated perfusion (HOPE) for fatty liver grafts in rats and humans. *J Hepatol* 2017.
 - [39] Jamieson RW, Zilvetti M, Roy D, Hughes D, Morovat A, Coussios CC, et al. Hepatic steatosis and normothermic perfusion-preliminary experiments in a porcine model. *Transplantation* 2011;92(3):289–295.
 - [40] Liu Q, Berendsen T, Izamis ML, Uygun B, Yarmush ML, Uygun K. Perfusion defatting at subnormothermic temperatures in steatotic rat livers. *Transpl Proc* 2013;45(9):3209–3213.
 - [41] Taba Taba Vakili S, Kailar R, Rahman K, Nezami BG, Mwangi SM, Anania FA, et al. Glial cell line-derived neurotrophic factor-induced mice liver defatting: a novel strategy to enable transplantation of steatotic livers. *Liver Transplant* 2016;22(4):459–467.
 - [42] Mwangi SM, Peng S, Nezami BG, Thorn N, Farris 3rd AB, Jain S, et al. Glial cell line-derived neurotrophic factor protects against high-fat diet-induced hepatic steatosis by suppressing hepatic PPAR- γ expression. *Am J Physiol Gastrointest Liver Physiol* 2016;310(2):G103–G116.
 - [43] Raigani S, Carroll C, Griffith S, Pendexter C, Rosales I, Deirawan H, et al. Improvement of steatotic rat liver function with a defatting cocktail during ex situ normothermic machine perfusion is not directly related to liver fat content. *PLoS One* 2020;15(5):e0232886.
 - [44] Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. *Am J Transpl* 2010;10(2):372–381.
 - [45] Monbaliu D, Liu Q, Libbrecht L, De Vos R, Vekemans K, Debbaud C, et al. Preserving the morphology and evaluating the quality of liver grafts by hypothermic machine perfusion: a proof-of-concept study using discarded human livers. *Liver Transplant* 2012;18(12):1495–1507.
 - [46] Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018;557(7703):50–56.
 - [47] Selzner M, Goldaracena N, Echeverri J, Kathis JM, Linares I, Selzner N, et al. Normothermic ex vivo liver perfusion using steen solution as perfusate for human liver transplantation: first North American results. *Liver Transpl* 2016;22(11):1501–1508.
 - [48] Watson CJE, Kosmolaptis V, Pley C, Randle L, Fear C, Crick K, et al. Observations on the ex situ perfusion of livers for transplantation. *Am J Transpl* 2018;18(8):2005–2020.
 - [49] Liu Q, Nassar A, Buccini L, Iuppa G, Soliman B, Pezzati D, et al. Lipid metabolism and functional assessment of discarded human livers with steatosis undergoing 24 hours of normothermic machine perfusion. *Liver Transpl* 2018;24(2):233–245.
 - [50] Boteon YL, Attard J, Boteon A, Wallace L, Reynolds G, Hubscher S, et al. Manipulation of lipid metabolism during normothermic machine perfusion: effect of defatting therapies on donor liver functional recovery. *Liver Transpl* 2019;25(7):1007–1022.
 - [51] Raigani S, Markmann JF, Yeh H. Rehabilitation of discarded steatotic livers using ex situ normothermic machine perfusion: a future source of livers for transplantation. *Liver Transpl* 2019;25(7):991–992.
 - [52] Nakamura M, Morizono S, Soejima Y, Yoshizumi T, Aishima S, Takasugi S, et al. Short-term intensive treatment for donors with hepatic steatosis in living-donor liver transplantation. *Transplantation* 2005;80(5):608–612.
 - [53] Clavien PA, Oberkofler CE, Raptis DA, Lehmann K, Rickenbacher A, El-Badry AM. What is critical for liver surgery and partial transplantation: size or quality? *Hepatology* 2010;52(2):715–729.
 - [54] Doyle A, Adeyi O, Khalili K, Fischer S, Dib M, Goldaracena N, et al. Treatment with Optifast reduces hepatic steatosis and increases candidacy rates for living donor liver transplantation. *Liver Transpl* 2016;22(9):1295–1300.
 - [55] Chung JH, Ryu JH, Yang KH, Choi BH, Park Y, Lee TB, et al. Efficacy and safety of weight reduction of the donor in hepatic steatosis for living donor liver transplantation. *Ann Transpl* 2020;25:e923211.
 - [56] Fujii Y, Kawamura N, Zaitsumi M, Watanabe M, Goto R, Kamiyama T, et al. Outcome of living-donor liver transplantation using grafts from donors treated for fatty liver. *Ann Transpl* 2020;25:e920677.
 - [57] Taneja S, Gupta S, Wadhawan M, Goyal N. Single-lobe living donor liver transplant in a morbidly obese cirrhotic patient preceded by laparoscopic sleeve gastrectomy. *Case Rep Transpl* 2013;2013:279651.