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Alterations in Gut Microbial Function Following Liver Transplant

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Abstract

Liver transplant (LT) improves daily function and ameliorates gut microbial composition. However, the effect of LT on microbial functionality, which can be related to overall patient benefit, is unclear and could affect the post-LT course.

Aims—To determine the effect of LT on gut microbial functionality focusing on endotoxemia, bile acid (BA), ammonia metabolism and lipidomics.

Method—We enrolled outpatient cirrhotic patients on the LT list and followed them till 6 months post-LT. Microbiota composition (Shannon diversity and individual taxa) and function analysis (serum endotoxin, urinary metabolomics and serum lipidomics, and stool BA profile) and cognitive tests were performed at both visits.

Results—We enrolled 40 patients (56±7 years, MELD 23). They received LT 6±3 months after enrollment and were re-evaluated 7±3 months post-LT with a stable course. A significant improvement in cognition with increase in microbial diversity, increase in autochthonous and decrease in potentially pathogenic taxa and reduced endotoxemia were seen post-LT compared to baseline. Stool BAs increased significantly post-LT and there was evidence of greater bacterial action (higher secondary, oxo and iso-BAs) post-LT although the levels of conjugated BAs remained similar. There was a reduced serum ammonia and corresponding rise in urinary phenylacetylglutamine (PAG) post-LT. There was an increase in urinary trimethylamine-N-oxide

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(TMAO), which was correlated with specific changes in serum lipids related to cell membrane products. The ultimate post-LT lipidomic profile appeared beneficial compared to the pre-LT.

Conclusions—LT improves gut microbiota diversity and dysbiosis which is accompanied by favorable changes in gut microbial functionality corresponding to bile acids, ammonia, endotoxemia, lipidomic and metabolomic profiles.

Keywords

Lipidomics; bile acids; Trimethylamine-*N*-oxide; endotoxin; metabolomics

Introduction

Liver transplantation (LT) is a life-saving procedure in end-stage liver disease, which is usually characterized by an uneventful post-LT recovery(1). However, a subset of patients has a post-operative course that is characterized by multiple infections that can impair the overall prognosis. There is evidence of unfavorable gut microbiota in end-stage liver disease or dysbiosis that is strongly related to negative outcomes in the pre-LT period(2, 3). In addition to an unfavorable gut microbial composition there is also evidence of impaired microbial functionality in advanced cirrhosis patients. This impaired functionality is reflected by a lower conversion from primary to secondary bile acids (BA), changes in ammonia metabolism, as well as changes in microbial-mammalian co-metabolites such as trimethylamine-*N*-oxide (TMAO)(4, 5). Since secondary BAs can suppress pathogens, their reduction in advanced cirrhosis is worrisome(6). TMAO is a microbial-mammalian co-metabolite that has been shown to link gut microbiota with atherogenesis and cardiovascular changes in the pre-transplant setting(4). Given the pro-atherogenic profile post-LT, the role of TMAO in this setting needs to be investigated. In addition, cirrhotic patients often have hepatic encephalopathy (HE), characterized by endotoxemia, inflammation and hyperammonemia(7). Hyperammonemia is likely due to gut microbial production exacerbated by liver dysfunction(8). This can potentially improve after LT in most cases but the role of microbiota function towards this is unclear.

While studies following patients from pre to the post-LT period have shown an improvement in gut microbial dysbiosis, functional changes have not been studied in detail(9, 10). The role of gut microbiota composition and functionality in the modulation of the post-LT course is important because probiotic therapy has been inconsistently shown to improve these outcomes(11).

We hypothesized that there would be a significant microbial functional change post-LT compared to their pre-LT baseline, which could be beneficial to the patients.

Experimental Procedures

Patients listed for deceased LT at Virginia Commonwealth University Medical Center and McGuire VA Medical Center between June 2011 and August 2015 were enrolled. We included patients who were between 21 and 65 years, were able to provide consent and give samples. We excluded those who were listed as status 1, listed for living donor transplant,

were unwilling to consent or provide samples or had HIV and those who were lost to follow-up. The protocol was approved by the Institutional Review Boards at both institutions.

After informed consent, patients underwent stool collection for gut microbiota and bile acid analysis. MELD score and venous ammonia were measured. A 24 hour dietary recall was performed in all patients, along with psychometric hepatic encephalopathy score testing (PHES)(7), which was used to diagnose minimal HE (MHE)(12). Stool bacterial DNA was extracted for multi-tagged sequencing using published techniques(2), while bile acids were evaluated using LC/MS techniques. Urine and serum were also collected during the same sitting for NMR metabolomics and lipidomics respectively (details below and in the supplementary data).

The included patients were followed until LT, death on the waiting list or delisting. For those who were transplanted, the transplant and immuno-suppression regimen details, post-transplant course including hospitalizations, rejection episodes and duration of hospital stay were recorded. The post-transplant visit was performed at after 6 months for patients who were alive and willing, and had not undergone a second transplant. This point was chosen post-transplant timeframe to avoid immediate and transient post-operative complications(13). At six months, all procedures performed at the baseline visit, were repeated.

Specialized testing and analyses

Stool microbiome was assayed using Multitag sequencing on an Ion Torrent PGM(2). The microbiota relative abundance was analyzed using Wilcoxon paired tests, and Linear Discriminant Analysis Effect Size (LEFSe) was used to compare the microbiota pre to the post-LT profiles (14, 15). Stool bile acid levels were analyzed using published LC/MS methods(16). Urine was collected the same day for metabolomics using NMR spectroscopy using published techniques(17). Serum lipidomics was performed using established methods(18). Detailed testing methods are in the supplementary section. Correlations and changes pre/post-LT using multiple-testing correction were performed between TMAO and lipidomics fractions. We also performed analysis of metabolites, diversity and the cognitive status pre/post-LT. Correlations between changes (delta, post-LT minus pre-LT) in Shannon diversity, individual bacterial phyla, BA profile and urinary metabolites were also performed using Pearson correlations.

Results

We enrolled 57 patients, of whom 5 died or were de-listed prior to LT, 4 were not able to provide all samples. The remaining 48 were transplanted, of whom 40 were seen post-LT. Of the remaining 8, 3 died within one month of LT, one required re-transplantation due to primary non-function and 4 were lost to follow-up.

The forty patients ultimately that underwent collections at both visits had a mean age of 56 ± 7 years, a mean MELD of 22.6 ± 6.3 . The majority (n=30) were Caucasian, and were men (n=35). The leading etiology of cirrhosis was hepatitis C (n=17), hepatitis C and alcohol (n=8) followed by non-alcoholic fatty liver disease (n=6), and others (n=9). Eleven patients

were on the list primarily for hepatocellular cancer. Per VCU protocol, all patients had a detailed cardiovascular work-up, including cardiac catheterization, showing minimal atherosclerotic disease before listing. Patients received LT 6 ± 3 months after enrollment. In the interim, of the 40, nine patients were hospitalized, six for infections (two *C.difficile*, two urinary tract infections with *Klebsiella* and *E.coli* and two with SBP without an organism isolated), and three for anasarca.

These patients were re-evaluated 7 ± 3 months post-LT for their second visit. The VCU immunosuppression protocol consists of steroids tapered over one month with peri-operative mycophenolate mofetil. Tacrolimus, started at day 3 is targeted to maintain 8-12 ng/ml levels within the first three months and 5-10 ng/ml after that period. Regular tacrolimus trough levels were monitored throughout and all patients were within the trough values that were recommended (10.5 ± 5.6 ng/ml average tacrolimus level prior to 6-month visit).

In the immediate post-LT period, there were infections seen in ten patients (*C.difficile* in five and biliary sepsis in four and surgical wound infection in 2; one person had both biliary sepsis and *C.difficile*). An additional two patients showed acute cellular rejection within 3 months post-LT treated with steroids, four required biliary interventions and two needed changing of the primary immunosuppression from tacrolimus to cyclosporine due to neuro-toxicity.

At the time of repeat evaluation, dietary recall and PHES testing were performed again. At that time none of the patients had recurrence of cirrhosis or had HCV eradicated. At the time of sample collection, there were no signs of active bacterial and fungal infection and the last use of antibiotics other than the recommended trimethoprim-sulfomethoxazole was at least 3 months prior. All patients were on calcineurin immunosuppression (tacrolimus in 38 and cyclosporine in 2 patients) with mycophenolate without sirolimus use. Details are shown in table 1.

There was a higher systolic and diastolic blood pressure and lower BMI after LT (Table 2). This was accompanied by recovery of the liver function and improvement in the hemoglobin, WBC count and renal function. Blood glucose levels were similar blood glucose before/after LT. The dietary pattern, including the ingestion of meat and eggs and the total caloric value remained similar before and after transplant. The relative proportion of protein intake was similar pre/post LT (17% calories from protein pre vs. 21% post LT).

Cognitive changes

There was a significant improvement in PHES score with a lowering of the proportion of MHE post-LT (Table 2).

Microbial composition and endotoxemia

There was a significant increase in Shannon Diversity indices and reduction in serum endotoxin after LT (Table 2). We found a significant reduction in the relative abundance of potentially pathogenic genera belonging to *Enterobacteriaceae* (*Escherichia*, *Shigella*, *Salmonella*) with a significant increase in potentially beneficial, autochthonous genera belonging to *Ruminococcaceae* and *Lachnospiraceae*, on LEFSe (Table 3) (9).

Bile acid changes

The fecal bile acid profile after LT was characterized by a significant increase in total BAs including primary BAs, specifically cholic acid (Table 4). When the bacterial actions on BAs were analyzed, post-LT there was a similar conjugated BAs but significantly higher secondary, iso and oxo BAs.

Urinary metabolomics

As shown in the figure 1, there were differences in urinary NMR spectroscopy as whole pre- and post-LT, including a significant increase in TMAO (increased 2.4 fold, $p=0.01$) and in phenylacetylglutamine (PAG) (increased 2.7 fold, $p=0.02$) after LT.

Ammonia changes

There was a significant reduction in venous ammonia (Table 2) and also an increase in urinary phenylacetylglutamine as mentioned above.

Lipidomics

We found a significant change in serum lipidomics after LT which was related to changes across several lipid classes, diacylglycerols, cholesterol esters, lyso-phosphethanolamine/phosphoethanolamine and sphingomyelin (all changes $p<0.05$ after multiple testing correction). Of these there was a decrease in serum PE36:1, DG35:5 and DG35:4 and an increase in serum DG44:11, CE20:4, SM39:2, LysoPE18:0 and LysoPE20:2 after LT (Figure 2A). Urinary TMAO showed significant positive correlations with LysoPE20:2 ($p=2.17E-02$), DG(44:11), and PC36:4 ($p=3.32E-02$) and negative correlations with PE36:1 ($p=2.12E-02$), DG35:5 ($p=4.24E-02$) and DG35:4 ($p=3.11E-02$) (Figure 2B).

Relationship between cognition, microbial function, and composition pre and post-LT

Correlations pre-LT were significant between PHES and PAG ($r=0.5$, $p=0.04$), relative abundance of *Firmicutes* ($r=0.42$, $p=0.05$). PAG was also correlated positively with TMAO ($r=0.6$, $p=0.02$) and negatively with *Proteobacteria* relative abundance ($r=-0.47$, $p=0.03$). TMAO was associated positively with primary ($r=0.7$, $p=0.008$) and total BA ($p=0.6$, $p=0.03$).

When delta changes were compared, delta PHES was negatively correlated with delta *Proteobacteria* ($r=-0.54$, $p=0.04$) and PAG ($r=-0.51$, $p=0.05$) and positively with *Firmicutes* ($r=0.6$, $p=0.01$). In addition delta PAG and delta TMAO were positively linked ($r=0.6$, $p=0.02$). Changes in total, primary and secondary BA were negatively linked with *Proteobacteria* (all $r=-0.54$, $p=0.01$) and positively with *Firmicutes* (all $r=0.49$, $p=0.05$). No significant correlations between Shannon diversity pre/post or delta were found with these metabolites or cognitive changes.

Discussion

Our results demonstrate that there is a beneficial change in the gut microbiota composition and functionality after successful LT. These changes are manifested by an improvement in microbiota diversity and beneficial autochthonous bacterial composition, as well as

improvement in fecal BA profile and serum lipidomics. Prior studies have shown an improvement in the gut microbial composition after LT are linked to brain function improvement(9, 19). We also found a beneficial impact on ammonia metabolism and resumption of methylamine synthesis.

These findings add our current knowledge where there is a dramatic clinical improvement in clinical and quality of life parameters in most cirrhotic patients after a successful liver transplant(9, 20). However, the post-LT stage is also complicated by the use of immunosuppressive, which can potentially modulate post-transplant outcomes(1). In addition, recurrence of the cirrhosis etiology and incomplete recovery from the sequelae from the complications such as hepatic encephalopathy can also occur in these patients(21).

In addition to the improvement in liver function and systemic hemodynamics, our results demonstrate an improvement of the gut microbial diversity. This was accompanied by a higher relative abundance of beneficial, autochthonous bacteria and suppression of potentially pathogenic gram-negative taxa post-LT, even with the use of immunosuppression and trimethoprim-sulfomethoxazole(22). Tacrolimus can decrease Firmicutes and increase Bacteroidetes, but we found the opposite trend post-LT(22). This highlights the importance of a functioning liver in the mediation of gut microbial improvement and also demonstrates that the gut microbial changes in cirrhosis are a potential result of liver insufficiency rather than an independent patho-physiological factor in disease causation(23). Moreover, these changes were accompanied by a significant reduction in endotoxemia, which can potentiate the systemic inflammatory milieu seen in cirrhotic patients(24, 25). This is a functional correlate of the reduction in gram-negative taxa such as those belonging to *Enterobacteriaceae*. In addition the potential improvements in the intestinal barrier due to increase in short-chain fatty acid producing *Lachnospiraceae* and *Ruminococcaceae* and reversal of portal hypertension, and improvement in the immune function could also contribute to the reduction in post-LT endotoxemia.

A major component of the gut-liver axis is the BAs, which are pleiotropic signaling molecules(26). Primary BAs (cholic and chenodeoxycholic acid) are synthesized from cholesterol in the liver and released into the duodenum conjugated with glycine or taurine. Bacterial modification in the intestine results in deconjugation (removal of the glycine/taurine) followed by 7 α dehydroxylation, forming secondary bile acids (deoxycholic and lithocholic acid)(27). Both secondary and primary BAs can further undergo a reversible transformation into oxo-BAs in the colonic bacteria. It makes them more hydrophilic and less toxic. Another bacterial transformation is the epimerization of BAs to form the non-toxic iso-bile acids. While most bacterial taxa are able to deconjugate BAs and form oxo-BAs, only selected microbes are able to perform 7 α dehydroxylation and conversion to iso-BAs(26). In cirrhotic patients, due to liver disease and intra/extra-hepatic cholestasis, there is a low BA production and secretion into the intestine(5). Furthermore, there is a reduction in secondary BAs due to lower autochthonous, beneficial bacteria that mediate the 7 α dehydroxylation reaction(28). Therefore the increase in total BAs with significant increase in secondary, iso and oxo-BAs is a reflection of the increase in *Firmicutes* and reduction in *Proteobacteria* post-LT which indicates a improvement of the intestinal milieu. This could

afford greater protection against nosocomial bacteria such as *C.difficile*, which are inhibited to a greater degree by secondary but not by primary BA moieties(6).

Another aspect of gut microbial function is the conversion of dietary amino acids and proteins into ammonia, which can be complicit in the development of hepatic encephalopathy(7). In healthy subjects, in addition to fixation of the ammonia into the urea cycle by the liver, there is a urinary excretion in the form of PAG. Post-LT, there was a reduction in venous ammonia that was accompanied by higher urinary PAG, indicating again an improvement in ammonia metabolism. Moreover as previously reported, this was associated with *Proteobacteria* and *Firmicutes* relative abundance changes (9) and also to urinary PAG level changes after LT.

While improvement in BAs, microbial diversity and endotoxemia point towards an overall improvement, there was also a significant increase in urinary TMAO. The mammalian microbial co-metabolite is produced through bacterial action on dietary carnitine and choline, and subsequent oxidation in the liver with flavin mono-oxygenases(4). TMAO is associated with negative cardiovascular outcomes and is associated with atherogenesis in non-cirrhotic individuals(29). Moreover in non-cirrhotic patients, TMAO can potentially interfere with hepatic BA synthesis(29). Given the significant increase in the fecal BA content despite this increase in TMAO, it is unlikely that there was a meaningful inhibition of hepatic BA synthesis in the newly transplanted livers. In cirrhotic patients, cognitive dysfunction is linked with TMAO levels(30, 31). Since the formation of TMAO requires a functioning microbiota and liver, the return to normal function after LT of both these organ systems make it difficult to separate the relative contribution of microbiota compared to the liver in the development of higher post-LT TMAO levels. Specifically bacterial taxa that are responsible for the conversion of primary to secondary BAs can also convert dietary choline into trimethylamine (TMA), i.e. Clostridium Cluster XIV(23, 32, 33). On the other hand there is evidence that members of *Enterobacteriaceae* possess the genes to covert dietary carnitine into TMA (32, 33). It is likely that the enhanced liver function was largely responsible for this increase given that dietary patterns, including red meat and egg intake, remained similar pre/post-LT and the bacterial profile demonstrated a mixed picture with respect to TMA converting taxa with a higher Clostridium cluster XIV and lower *Enterobacteriaceae* post-LT.

To better define the relationship of TMAO and potential atherogenesis, serum lipidomic changes were studied. Lipid profiles after LT showed a trend towards normalization with the profile changing from shorter-chain fatty acids and markers of cell membrane damage pre-LT to that of longer-chain fatty acids and intact cell membrane markers post-LT. Specific changes in the lipidomics analysis reveal several interesting results. The choline degradation pathway with TMAO generation represents a unique additional nutritional contribution to the pathogenesis of cardiovascular disease that involves phosphatidylcholine and choline metabolism, an obligate role for the intestinal microbial community, and regulation of surface expression levels of macrophage scavenger receptors known to participate in the atherosclerotic process(34). Choline is a constituent of phosphatidylcholine and it is essential for the structural integrity of cell membranes, acetylcholine synthesis, cell-membrane signaling and methyl-group metabolism. Changes in diacylglycerol post-LT are

relevant since fish oils, those rich in omega-3 fatty acids (DHA and EPA) in particular, help to prevent the progression of atherosclerosis by inhibiting the development of plaques and blood clots at lesion sites(35). Dietary DAG might be useful for the regression of atherosclerosis compared with TAG, which has a similar fatty acid composition, suggesting that the effects of DAG are related to the acylglycerol structure(36). Sphingomyelin change with LT is important because, in addition to being a major lipid component of plasma and cell membranes, it is an independent risk factor for coronary artery disease(37). Oxidized cholesterol (oxysterols) enhances the production of sphingomyelin, a phospholipid found in the cellular membranes of the coronary artery. This increases the sphingomyelin content in the cell membrane, which in turn increases the risk of arterial calcification(37). The redistribution of cholesteryl esters, triglycerides, and, to a lesser extent, phospholipids in plasma could be achieved by glycoprotein which is secreted by liver, and circulates in plasma, which could also modulate the risk of atherosclerosis(38-40).

Ultimately beneficial lipidomic markers were significantly correlated with TMAO increase post-LT. At this stage post-LT, it is unlikely to see de novo cardiovascular consequences and the selection of our patients ensured that those with an eventful post-operative course had stabilized months before inclusion. Therefore the increase in TMAO at this early post-LT stage is likely a manifestation of return to relative normalcy of the liver, given that it was positively linked with other beneficial changes such as increase in urinary PAG, and decrease in gut *Proteobacteria* relative abundance. These changes are even more relevant since all pre-LT patients are cleared from a cardiovascular perspective before listing. Given that most post-LT cardiovascular disease occurs several years later, further longitudinal follow-up is required to determine if this TMAO increase persists and is related to atherogenic events further along the post-LT course.

We only included stable patients after 6 months of the transplant, and excluded those with recent rejection and infectious events. This implies that these events could not be prognosticated by the microbial functional changes. However, our aim was to determine the reversibility of microbial functional changes post-LT and needed to avoid confounding factors. The study is limited by the relatively small number of patients with NAFLD, who could indeed have a worse post-LT atherogenic course. In addition, none of our HCV patients underwent eradication pre or post-LT given the timeframe of recruitment, which could potentially change these results given the effect of HCV on lipidomics(41). While individual taxa were linked as expected to metabolites and outcomes, there was no significant relationship with Shannon diversity. This could be due to the relative narrow skewed diversity pre-LT and also reflects that diversity is only one aspect of the complex microbial analysis. We also performed the sample analysis at a time point beyond any bacterial infections such as *C.difficile*, therefore this data cannot be leveraged to analyze that risk. Our sample size is limited, which did not allow for separation of differing cirrhosis etiologies such as NAFLD pre and post-LT. We do not have healthy control data for comparison but given the significant co-morbid conditions, the patients serving as their own control was considered appropriate. However, the microbiota composition, BA profiles, endotoxin levels and lipidomic changes approximate the healthy population values(2, 5, 9, 42).

We conclude that after successful liver transplant there is a significant beneficial change in gut microbial function that spans endotoxin synthesis, ammonia, bile acid modulation and methylamine metabolism. Cumulatively, these changes indicate a return to normal gut microbial composition and function in diverse aspects within six months of a successful liver transplant and underline the central role of the liver in determining the gut microbial function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

LT	liver transplant
BA	bile acids
HE	hepatic encephalopathy
TMAO	trimethylamine-N-oxide
TMA	Tri-methylamine
LEFSe	Linear Discriminant Analysis Effect Size
PAG	phenylacetyl-glutamine
DAG	diacylglycerol,

References

1. Martin P, DiMartini A, Feng S, Brown R Jr, Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology*. 2014; 59:1144–1165. [PubMed: 24716201]
2. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol*. 2014; 60:940–947. [PubMed: 24374295]
3. Chen Y, Guo J, Qian G, Fang D, Shi D, Guo L, Li L. Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality. *J Gastroenterol Hepatol*. 2015
4. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472:57–63. [PubMed: 21475195]
5. Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol*. 2013; 58:949–955. [PubMed: 23333527]

6. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature*. 2015; 517:205–208. [PubMed: 25337874]
7. Vilstrup H, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. *Hepatology*. 2014; 60:715–735. [PubMed: 25042402]
8. Shawcross DL, Davies NA, Williams R, Jalan R. Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis. *J Hepatol*. 2004; 40:247–254. [PubMed: 14739095]
9. Bajaj JS, Fagan A, Sikaroodi M, White MB, Sterling RK, Gilles H, Heuman D, et al. Liver transplant modulates gut microbial dysbiosis and cognitive function in cirrhosis. *Liver Transpl*. 2017; 23:907–914. [PubMed: 28240840]
10. Doycheva I, Leise MD, Watt KD. The Intestinal Microbiome and the Liver Transplant Recipient: What We Know and What We Need to Know. *Transplantation*. 2016; 100:61–68. [PubMed: 26647107]
11. Zhang Y, Chen J, Wu J, Chalson H, Merigan L, Mitchell A. Probiotic use in preventing postoperative infection in liver transplant patients. *Hepatobiliary Surg Nutr*. 2013; 2:142–147. [PubMed: 24570932]
12. Allampati S, Duarte-Rojo A, Thacker LR, Patidar KR, White MB, Klair JS, John B, et al. Diagnosis of Minimal Hepatic Encephalopathy Using Stroop EncephalApp: A Multicenter US-Based, Norm-Based Study. *Am J Gastroenterol*. 2016; 111:78–86. [PubMed: 26644276]
13. Pillai AA, Levitsky J. Overview of immunosuppression in liver transplantation. *World J Gastroenterol*. 2009; 15:4225–4233. [PubMed: 19750565]
14. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011; 12:R60. [PubMed: 21702898]
15. <http://qiime.org/tutorials/tutorial.html>. In.
16. Muto A, Takei H, Unno A, Murai T, Kurosawa T, Ogawa S, Iida T, et al. Detection of Delta4-3-oxo-steroid 5beta-reductase deficiency by LC-ESI-MS/MS measurement of urinary bile acids. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2012; 900:24–31.
17. Bajaj JS, Cox JJ, Betrapally NS, Heuman DM, Schubert ML, Ratneswaran M, Hylemon PB, et al. Systems Biology Analysis of Omeprazole Therapy in Cirrhosis Demonstrates Significant Shifts in Gut Microbiota Composition and function. *Am J Physiol Gastrointest Liver Physiol*. 2014
18. Xu J, Casas-Ferreira AM, Ma Y, Sen A, Kim M, Proitsi P, Shkodra M, et al. Lipidomics comparing DCD and DBD liver allografts uncovers lysophospholipids elevated in recipients undergoing early allograft dysfunction. *Sci Rep*. 2015; 5:17737. [PubMed: 26635289]
19. Wu ZW, Ling ZX, Lu HF, Zuo J, Sheng JF, Zheng SS, Li LJ. Changes of gut bacteria and immune parameters in liver transplant recipients. *Hepatobiliary Pancreat Dis Int*. 2012; 11:40–50. [PubMed: 22251469]
20. Campagna F, Biancardi A, Cillo U, Gatta A, Amodio P. Neurocognitive-neurological complications of liver transplantation: a review. *Metab Brain Dis*. 2010; 25:115–124. [PubMed: 20204483]
21. Campagna F, Montagnese S, Schiff S, Biancardi A, Mapelli D, Angeli P, Poci C, et al. Cognitive impairment and electroencephalographic alterations before and after liver transplantation: what is reversible? *Liver Transpl*. 2014; 20:977–986. [PubMed: 24809329]
22. Ling Q, Xu X, Wang B, Li L, Zheng S. The Origin of New-Onset Diabetes After Liver Transplantation: Liver, Islets, or Gut? *Transplantation*. 2016; 100:808–813. [PubMed: 26910326]
23. Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids, and gut microbiota: Unraveling a complex relationship. *Gut Microbes*. 2013; 4
24. Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis*. 2008; 28:26–42. [PubMed: 18293275]
25. Lin CY, Tsai IF, Ho YP, Huang CT, Lin YC, Lin CJ, Tseng SC, et al. Endotoxemia contributes to the immune paralysis in patients with cirrhosis. *J Hepatol*. 2007; 46:816–826. [PubMed: 17328986]

26. Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab.* 2016; 24:41–50. [PubMed: 27320064]
27. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res.* 2006; 47:241–259. [PubMed: 16299351]
28. Bajaj JS, Betrapally NS, Gillevet PM. Decompensated cirrhosis and microbiome interpretation. *Nature.* 2015; 525:E1–2. [PubMed: 26381988]
29. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med.* 2013; 19:576–585. [PubMed: 23563705]
30. Bajaj JS, Gillevet PM, Patel NR, Ahluwalia V, Ridlon JM, Kettenmann B, Schubert CM, et al. A longitudinal systems biology analysis of lactulose withdrawal in hepatic encephalopathy. *Metab Brain Dis.* 2012; 27:205–215. [PubMed: 22527995]
31. Jimenez B, Montoliu C, MacIntyre DA, Serra MA, Wassel A, Jover M, Romero-Gomez M, et al. Serum metabolic signature of minimal hepatic encephalopathy by (1)H-nuclear magnetic resonance. *J Proteome Res.* 2010; 9:5180–5187. [PubMed: 20690770]
32. Romano KA, Vivas EI, Amador-Nogues D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. *MBio.* 2015; 6:e02481. [PubMed: 25784704]
33. Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. *Microbiome.* 2017; 5:54. [PubMed: 28506279]
34. Ierardi E, Sorrentino C, Principi M, Giorgio F, Losurdo G, Di Leo A. Intestinal microbial metabolism of phosphatidylcholine: a novel insight in the cardiovascular risk scenario. *Hepatobiliary Surg Nutr.* 2015; 4:289–292. [PubMed: 26312245]
35. Connor SL, Connor WE. Are fish oils beneficial in the prevention and treatment of coronary artery disease? *Am J Clin Nutr.* 1997; 66:1020S–1031S. [PubMed: 9322583]
36. Ota N, Soga S, Hase T, Tokimitsu I, Murase T. Dietary diacylglycerol induces the regression of atherosclerosis in rabbits. *J Nutr.* 2007; 137:1194–1199. [PubMed: 17449581]
37. Kummerow FA. Interaction between sphingomyelin and oxysterols contributes to atherosclerosis and sudden death. *Am J Cardiovasc Dis.* 2013; 3:17–26. [PubMed: 23459228]
38. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2003; 23:160–167. [PubMed: 12588754]
39. Barter P, Rye KA. Cholesteryl ester transfer protein inhibition to reduce cardiovascular risk: Where are we now? *Trends Pharmacol Sci.* 2011; 32:694–699. [PubMed: 22088767]
40. Rudel LL, Shelness GS. Cholesterol esters and atherosclerosis—a game of ACAT and mouse. *Nat Med.* 2000; 6:1313–1314. [PubMed: 11100106]
41. Qu F, Zheng SJ, Wu CS, Jia ZX, Zhang JL, Duan ZP. Lipidomic profiling of plasma in patients with chronic hepatitis C infection. *Anal Bioanal Chem.* 2014; 406:555–564. [PubMed: 24253410]
42. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, et al. A core gut microbiome in obese and lean twins. *Nature.* 2009; 457:480–484. [PubMed: 19043404]

Figure 1. Urinary Metabolomic Changes using NMR spectroscopy pre compared to post liver transplant (LT)

A: Principal component analysis shows separation of pre-LT (●) and post-LT (●) groups (class separation 0.61)

B: Relative levels of trimethylamine-*N*-oxide increased significantly after LT compared to pre-LT levels. Data presented as mean and 95% CI with outliers separately identified

C: Relative levels of phenylacetylglutamine increased significantly after LT compared to pre-LT levels. Data presented as mean and 95% CI with outliers separately identified

Figure 2. Serum Lipidomic changes using LC/MS pre compared to post liver transplant (LT)

A: Relative Levels of 6 lipids (including DG44:11, SM39:2, CE20:4 and LysoPE20:2) increased after liver transplant, whilst 3 lipids (DG35:4, DG35:5 and PE36:1) decreased. Data presented as mean and 95% CI with individual values. DG: diacylglycerol, SM: sphingomyelin, PE: phosphoethanolamine, CE: cholesteryl ester.

B: OPLS-Y model shows how lipids (X axis) interacted with TMAO (Y axis) pre and post-LT. The loading plot shows that 305 serum lipids features increased and 289 serum lipid features decreased with increased TMAO levels. The blue point indicates TMAO levels, green points indicate lipids that did not change significantly, red lipids indicate positive while blue lipids are negatively correlated.

Table 1

Characteristics of patients in the post-LT setting at the time of sample collection

Variable (n=40)	Variable
Age	56±7 years
Male Gender	35
Race (Caucasian/African-American/Hispanic)	30/6/4
Cirrhosis etiology (HCV/Alcohol/HCV+Alcohol/NASH/other)	17/2/8/6/7
Listing for hepatocellular cancer	11
HCC recurrence	0
Diabetes	23
Treatment for hyperlipidemia	5
On Dialysis	2
Infections after LT	10
Rejection episodes	2 (both treated with steroids)
Immunosuppressive regimen	
Tacrolimus	38
Cyclosporine	2
Mycophenolate mofetil	40
Sirolimus	0
Other medications	
Proton pump inhibitors	37
Trimethoprim-sulfamethoxazole	40

Table 2

Comparison between Pre and Post-Liver transplant

	Pre-Liver transplant	Post-Liver transplant	P value
BMI	31.6±5.5	29.1±4.7	0.001
Systolic BP (mmHg)	117.9±16.3	132.2±26.8	0.01
Diastolic BP (mmHg)	68.9±8.9	83.1±9.9	<0.001
Hemoglobin (g/dl)	11.2±2.2	12.0±2.4	0.04
WBC count (/mm ³)	5.3±2.8	4.1±2.6	0.03
Serum glucose (mg/dL)	126.5±47.4	117.6±29.3	0.15
INR	1.5±0.40	1.2±0.4	0.001
Serum sodium (mmol/L)	135.6±5.3	140.7±2.5	<0.001
Serum creatinine (mg/dL)	1.4±0.5	1.3±0.9	0.07
Serum albumin (g/dL)	3.2±0.7	3.9±0.5	<0.01
Serum bilirubin (mg/dL)	3.2±2.9	0.8±0.3	<0.001
Serum AST (IU)	89.9±50.1	44.6±42.2	<0.001
Serum ALT (IU)	66.1±53.0	49.9±43.0	0.07
Serum Alkaline phosphatase (IU)	211.9±239.8	148.7±105.7	0.15
Venous ammonia	95±38	24±14	0.001
Median PHES score	−4	−1	0.02
Minimal Hepatic Encephalopathy	24	11	0.003
24 hour caloric intake	2352±649	2409±901	0.26
Shannon Microbial Diversity	1.6±0.7	2.1±0.7	0.001
Serum Endotoxin (EU/ml)	0.78±0.3	0.09±0.4	0.001

All data presented as mean±SD unless mentioned otherwise. pre vs. post-transplant comparisons performed using paired t-tests, a higher composite PHES indicates better cognition

Table 3

LEFSe results of gut microbiota pre and post Liver transplant

Phylum_Family_Genus	Higher in	LDA value
<i>Proteobacteria_Enterobacteriaceae_Shigella</i>	Pre-LT	4.12
<i>Proteobacteria_Enterobacteriaceae_Escherichia</i>	Pre-LT	3.94
<i>Actinobacteria_Bifidobacteriaceae_Bifidobacterium</i>	Pre-LT	3.87
<i>Proteobacteria_Enterobacteriaceae_Salmonella</i>	Pre-LT	2.98
<i>Firmicutes_ClostridialesIncertaeSedisXI_Desulfatibacter</i>	Post-LT	2.45
<i>Firmicutes_Ruminococcaceae_ClostridiumIV</i>	Post-LT	2.67
<i>Firmicutes_ClostridialesIncertaeSedisXIII_Anaerovorax</i>	Post-LT	3.10
<i>Firmicutes_Ruminococcaceae_Oscillibacter</i>	Post-LT	3.94
<i>Firmicutes_Lachnospiraceae_Anaerostipes</i>	Post-LT	2.14
<i>Firmicutes_Lachnospiraceae_ClostridiumXIVb</i>	Post-LT	3.75
<i>Firmicutes_Lachnospiraceae_Blautia</i>	Post-LT	4.35
<i>Bacteroidetes_Rikenellaceae_Alistipes</i>	Post-LT	4.23
<i>Firmicutes_Streptococcaceae_Streptococcus</i>	Post-LT	4.01
<i>Firmicutes_Clostridiaceae_Butyricicoccus</i>	Post-LT	3.78
<i>Firmicutes_Lachnospiraceae_Roseburia</i>	Post-LT	3.86
<i>Firmicutes_Clostridiaceae_ClostridiumXIVa</i>	Post-LT	3.67
<i>Proteobacteria_Sutterellaceae_Suterella</i>	Post-LT	3.83
<i>Firmicutes_Lachnospiraceae_Dorea</i>	Post-LT	3.70
<i>Firmicutes_ClostridialesIncertaeSedisXI_Sporanaerobacter</i>	Post-LT	3.65
<i>Proteobacteria_Desulfovibrionales_Bilophila</i>	Post-LT	2.45

LDA: linear discriminant analysis, LEFSe: LDA effect size

Table 4

Fecal Bile Acid profile changes after LT

Micromoles/gm of stool	Pre-LT	Post-LT
<i>Total BAs</i>	3.44±4.62	10.43±9.78 *
<i>Total Primary BAs</i>	1.73±2.21	4.31±5.8 *
Cholic acid	0.41±0.64	1.85±2.1 *
Chenodeoxycholic acid	0.24±0.45	0.47±0.87
<i>Total Secondary BAs</i>	1.07±2.82	6.23±7.10 *
Deoxycholic acid	0.56±1.34	2.65±3.3 *
Lithocholic acid	0.54±0.95	1.25±0.95 *
<i>Total conjugated BAs</i>	0.29±0.54	0.12±0.16
Total conjugated Primary BAs	0.01±0.02	0.07±0.14
Total conjugated Secondary BAs	0.28±0.54	0.04±0.04
<i>Total Oxo-BAs</i>	0.47±0.74	2.16±2.72 *
Total Primary Oxo-BAs	0.37±0.62	2.01±2.79 *
Total Secondary Oxo-BAs	0.09±0.10	0.15±0.11 *
<i>Total Iso-BAs</i>	0.44±0.57	1.37±1.7 *
Total Primary Iso-BAs	0.0±0.0	0.92±0.18
Total Secondary Iso-BAs	0.45±0.67	1.27±1.7 *

*
p<0.05 pre vs post-transplant using paired t-tests