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REVIEW

# Molecular pathological epidemiology in diabetes mellitus and risk of hepatocellular carcinoma

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## Abstract

Molecular pathological epidemiology (MPE) is a multidisciplinary and transdisciplinary study field, which has emerged as an integrated approach of molecular pathology and epidemiology, and investigates the relationship between exogenous and endogenous exposure factors, tumor molecular signatures, and tumor initiation, progression, and response to treatment. Molecular epidemiology broadly encompasses MPE and conventional-type molecular epidemiology. Hepatocellular carcinoma (HCC) is the third most common cause of cancer-associated death worldwide and remains as a major public health challenge. Over the past few decades, a number of epidemiological studies have demonstrated that diabetes mellitus (DM) is an established independent risk factor for HCC. However, how DM affects the occurrence and development of HCC remains as yet unclearly understood. MPE may be a promising approach to investigate the molecular mechanisms of carcinogenesis of DM in HCC, and provide some useful insights for this pathological process, although a few challenges must be overcome. This review highlights the recent advances in this field, including: (1) introduction of MPE; (2) HCC, risk factors, and DM as an established independent risk factor for HCC; (3) molecular pathology, molecular epidemiology, and MPE in DM and HCC; and (4) MPE studies in DM and risk of HCC. More MPE studies are expected to be performed in future and I believe that this field can provide some very important insights on the molecular mechanisms, diagnosis, personalized prevention and treatment for DM and risk of HCC.

**Key words:** Diabetes mellitus; Molecular pathological epidemiology; Hepatocellular carcinoma; Risk factor; Molecular mechanism



**Core tip:** Diabetes mellitus (DM) is an established independent risk factor for hepatocellular carcinoma (HCC); however, how DM affects the occurrence and development of HCC remains as yet unclearly understood. Molecular pathological epidemiology (MPE) may be a promising approach to investigate the molecular mechanisms of carcinogenesis of DM in HCC, and provide some

useful insights for this pathological process. This review highlights the recent advances in this field and more MPE studies are expected to be performed for this question in future.

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## INTRODUCTION

Molecular pathology examines the expression of molecular markers within bodily fluids, tissues or organs, and focuses on the diagnosis and studies of diseases, such as tumors[1,2]. Epidemiology is focused upon the studies of distributions and determinants of diseases and health conditions in specific populations[3,4]. Molecular pathological epidemiology (MPE) is a multidisciplinary and transdisciplinary study field, which has emerged as an integrated approach of molecular pathology and epidemiology, and investigates the relationship between tumour molecular markers, exposure of endogenous and exogenous factors, and development, progression and prognosis of tumors[5-8]. Molecular epidemiology broadly encompasses MPE and conventional-type molecular epidemiology.

In MPE, researchers investigate the relationships between: (1) changes of extracellular or cellular molecules (disease molecular signatures); (2) genetic, dietary, environmental and lifestyle factors; and (3) development and progression of diseases, such as tumors[6]. In 2010, Professor Shuji Ogino and Professor Meir Stampfer[5] were the first to introduce the concept of MPE. They consolidated this concept mainly based on the researches of colorectal cancer (CRC), particularly the prototypical study in the evolving field of MPE, which was conducted by Professor Peter T Campbell and others[9].

This case-control study of Campbell *et al*[9] was conducted to determine the relationships between CRC microsatellite instability (MSI) status, risk of CRC, and human body mass index (BMI). The results showed that an increased CRC risk was found in those patients with a high BMI; however, this risk of CRC was associated with the MSI status. For patients with MS-stable, the adjusted odds ratio (OR) was 1.38 and 95%CI was 1.24-1.54, for an increment of 5 kg/m$^2$ of BMI; for patients with MSI-low, the OR was 1.33 and 95%CI was 1.04-1.72; however, for patients with MSI-high tumours, the value of OR and 95%CI were 1.05 and 0.84-1.31, respectively[9]. The authors concluded that the relationship between the high BMI and increased CRC risk was related to the tumor MSI status[9]. According to the concept and principle of MPE, this prototypical study addressed the relationship between exposure factor (high BMI), molecular change (CRC MSI status) and tumor initiation (risk of CRC)[5,10].

MPE addresses two questions: (1) the association of particular exposure factors with specific molecular changes; and (2) the interaction of particular exposure factors with specific molecular changes to affect development, progression and prognosis of tumors. The typical research of cancer MPE is used to examine the relationship between exposure factors and risk of tumors according to the status of tumor signatures[9,10]. Cancer MPE techniques and studies can help us understand the carcinogenesis of certain exposure factors, through the examination of molecular pathological signatures associated with initiation and progression of tumors, and the exposures[5,6].

## HEPATOCELLULAR CARCINOMA AND RISK FACTORS

Hepatocellular carcinoma (HCC) has been confirmed as the third leading cause globally, among all the cancer-related deaths[10-12]. For primary liver cancers, more than 80% are HCC and the incidence rate annually of HCC is 4.9 per 100000 persons. Although some advances have been gained in the diagnosis and treatment of HCC, the prognosis remains very poor. Similarly, the annual mortality rate remains very high and HCC has also been ranked as one of the most lethal cancers[13].

With the using and popularization of hepatitis B virus (HBV) vaccination, the improvement of people's living standard and life style, and advancement of early diagnosis and treatment of premalignant lesions, the incidence of HCC had been anticipated to be decreased. However, the incidence rate of HCC has already been found to be increased significantly in the past thirty years in some countries, including the United States, China and Japan[14,15]. For example, during the period of 1981-1983 in the United States, the age adjusted incidence rate was 1.3 per 100000; however, this rate increased to 3.0 per 100000 during the period of 1996-1998[14,15]. Although more than fifty percent of this increase has been attributed to hepatitis virus C (HCV), other hepatitis viruses and alcoholic liver disease[16], the reason remains as unclear.

The identified risk factors of HCC include liver cirrhosis, HBV, HCV, heavy alcoholic consumption, alfatoxin exposure, non-alcoholic steatohepatitis, positive family history, male sex, and increasing age[17-19]. Over the past few decades, a number of epidemiological studies have demonstrated that diabetes mellitus (DM) is an established independent risk factor for HCC[12,20-23].

## DM AS AN ESTABLISHED INDEPENDENT RISK FACTOR FOR HCC

In the year of 1986, for the first time, Lawson *et al*[24] proposed accidentally the positive association of DM with HCC. The authors observed that, in Western Europe, the incidence rate of primary liver cancers was increased, and deduced that this increase might in part be associated

with the induction of hepatic microsomal enzyme caused by long-term usage of some drugs. On the basis of this assumption, the authors designed and performed an observational case-control study, which included 105 patients with HCC and long-term drug use, and 105 age and sex-matched patients with colorectal tumors and with fractures of femur[24]. Surprisingly, the results demonstrated that compared to the control group, the HCC group patients had four-fold excess of diabetic cases, and this association was independent of those pre-existing diseases, for example viral hepatitis, alcoholic cirrhosis and haemochromatosis[24]. The relationship between DM and HCC was proposed clearly although some limitations could not be avoided.

Following the publication of this study, only a few researches attempted to elucidate the association of diabetes with HCC in the next more than ten years; however, over the past more than one decade, more and more researches have been designed and performed to address this relationship[21,25-27]. Earlier epidemiologic studies showed inconsistent findings relating to the association of DM with HCC[21,28-30] whereas more and more recent studies have identified DM as an established independent risk factor for HCC, especially two prospectively large-scale population-based cohort studies[31,32]. In 2008, a review published in the journal of LANCET ranked diabetes as the fourth risk factor for HCC, following cirrhosis, viral hepatitis B and C, and non-alcoholic steatohepatitis[17].

Of the two prospectively large-scale population-based cohort studies[31,32], one was performed in the Sweden, which used the Swedish In-patient Register and included 153852 patients diagnosed with diabetes during the period between 1965 and 1983[31]. The patients were followed up through December 31, 1989. The authors identified those incident cases of cancer using the database and excluded those patients who were diagnosed with liver cancers during the first year of follow-up. The results showed that an increased risk of developing primary liver cancers was found in the diabetic patients (standardized incidence ratio, SIR = 4.1; 95%CI: 3.8-4.5). After exclusion of those concomitant diseases which have been associated with HCC, for example hepatitis, cirrhosis, and alcoholism, the persistence of an approximately threefold excess risk was observed[31].

The conclusion from the Swedish study was supported by another followed cohort study conducted in the United States[32], which was performed by doctors in the Department of Veterans Affairs. In this study, the authors also used the computerized records to identify all the patients with a hospital discharge diagnosis of DM in the period from 1985 to 1990, and matched randomly three patients without DM for every diabetic patient. Follow-up of these patients was taken through December 31, 2000. The major strength of this study was the strict inclusion and exclusion criteria and they were pre-determined perfectly on the basis of our current knowledge. The authors decided and used three periods, including: (1) the period dating back to 1980; (2) the period of index hospitalization; and (3) the period of the first year of follow-up. During these three above-mentioned periods, those patients with all kinds of liver diseases, abnormal liver function tests, alcoholism, or other identified risk factors for HCC, such as HBV and HCV, had been excluded from the study population[32]. The authors concluded that among men with diabetes, the risk of HCC was increased, which was not associated with demographic features, viral hepatitis, cirrhosis, and alcoholic liver disease.

The recently published systematic review in this field was designed to evaluate the impact of DM on the risk of HCC among patients with HCV infection[33]. This research included seven articles and all of them were conducted in Asian cohorts, including three studies from Taiwan, China, and four from Japan[34-40]. Among these studies, six were observational cohorts and six studies were of good quality. The results showed that a significantly increased risk of HCC was associated with DM in five of these seven studies and the effect sizes ranged from HR = 1.73 (95%CI: 1.30-2.30) to RR = 3.52 (95%CI: 1.29-9.24)[33].

## MOLECULAR PATHOLOGY, MOLECULAR EPIDEMIOLOGY AND MPE IN DIABETES

### *Molecular pathology in diabetes*

Pathology is an important constituent part of diagnostics, modern medicine and causal studies of diseases, which focuses upon four research fields of diseases: Etiology (causes), pathogenesis (mechanisms of development and progression), morphologic alterations (structural changes of cells, tissues and organs), and clinical manifestations (consequences of alterations)[41,42]. Molecular pathology (MP), whose focus is the examination of molecular signatures, has some similar aspects of practice to other disciplines, such as anatomic pathology, genetics, biochemistry, proteomics, molecular biology, and clinical pathology. Application of modern MP often encompasses three components: (1) exploration and confirmation of predictive molecular biomarkers for development, progression and treatment of diseases; (2) development of genetic and molecular approaches for diagnosis and classification of human diseases; and (3) susceptibility of individuals of different genetic constitution to particular disorders.

Molecular pathological studies in diabetes provide better insight into the etiology. For example type 1 or insulin-dependent diabetes, at least 20 genes have been identified and the dominant susceptibility locus maps to the major histocompatibility complex[43,44]. Major areas of MP research include environmental trigger factors, modification of the beta cells, infiltration of the islets by immuno-inflammatory cells, and autoimmune-mediated destruction of the beta cells. For T2DM, since the early genome-wide association studies (GWAS) in 2007, hundreds of genetic loci have been identified. Elucidating the pathology of DM at the molecular level is very important for developing innovative, personalized, and evidence-based treatments[45,46].

From the viewpoint of MP of DM in cancers, disruption of homeostatic glucose metabolism has been significantly associated with the malignant cellular transformation and tumor progression. In addition, the pathophysiology of disrupted glucose-insulin axis pathways of DM has been understood deeply at the subcellular level, thanking for the recent advances in biochemical and molecular technology. They may be useful for better understanding of the malignant cellular transformation, such as HCC.

### *Molecular epidemiology in diabetes*

In the late 20th century, with great advancement of biomedical sciences, a number of molecular signatures or biomarkers were identified as predictors of disease initiation, progression, and response to treatment, including diabetes and tumors. Since the identification of these molecular signatures, molecular epidemiology has evolved and been broadly named, which refers to the branch of epidemiology, where investigators examine these signatures in special study populations and its interaction with environmental, lifestyle or dietary factors, to perform the causal studies of diseases with aetiological factors[6,10]. Since the 2000s, GWAS has been commonly performed to identify genetic risk factors for diseases and health conditions[47,48].

Molecular epidemiology in diabetes is focused upon the contribution of possible environmental and genetic risk factors, to the distributions and determinants of DM within families and across populations, at the molecular level. For example, a number of molecular epidemiological studies demonstrate that some growth factors, including insulin, growth hormone, insulin-like growth factors and their binding proteins, may be important in the pathophysiological processes of T2DM[49]. In addition, many physiological changes have been associated with T2DM, including insulin resistance and hyperinsulinemia, increased estrogen levels, increased inflammatory cytokines such as tumor necrosis factor (TNF)-α, and interleukin (IL)-6, as well as altered levels of circulating adipokines[50]. It is well known that some of these molecular signatures and physiological changes may contribute to the development of cancers. Therefore, the relationship between DM and cancers, such as HCC, may be built *via* these molecular signatures or biomarkers.

### *MPE in diabetes*

MPE emerges as an integrated approach of molecular pathology and epidemiology, and investigates the relationship between risk factors, molecular signatures, and development and progression of diseases[10]. According to the concept and principle of MPE, the MPE approaches can also be used in non-neoplastic diseases, such as DM[51]. Although great advancements have been made in molecular pathology and molecular epidemiology, and a lot of molecular signatures have been associated with DM, no MEP studies in DM have been performed and the reason may be deduced that no identified risk factors are found for DM, such as HBV or HCV for HCC.

However, a few MPE studies had been performed when DM was treated as the risk factor for other diseases, such as cancers and coronary artery lesions, before the proposal and/or use of the concept of MPE, and they were conducted usually under the umbrella of molecular epidemiology. For example, one MPE study was designed to determine the relationship between 8-oxoguanine glycosylase (hOGG1) Ser326Cys gene polymorphism and coronary artery lesions in patients with DM[52]. In this study, 323 diabetic patients were included and the results showed that hOGG1 Ser326Cys polymorphism was correlated with coronary artery lesions in patients with DM, and Cys/Cys genotype may be associated with the more severity of lesions[52].

## MOLECULAR PATHOLOGY, MOLECULAR EPIDEMIOLOGY AND MPE IN HCC

### *Molecular pathology in HCC*

For human cancers, including CRC and HCC[53-55], molecular pathology is commonly used in the diagnosis and classification. Traditional molecular pathology studies are focused upon the molecular characteristics in cancer cells to improve our understanding of tumor cell behavior and carcinogenic processes[1,6,10]. However, human cancers are complex multifactorial diseases. Recent studies suggest that cancers should be classified based on salient clinical and pathologic features as well as on molecular fingerprints, which has been named "molecular classification", because of the premise that tumors with similar characteristics share common pathogenic mechanisms and progression patterns, despite each tumor undergoing its own unique neoplastic transformation[5,6,56]. Molecular classification is helpful to better understand the pathogenesis of tumors, predict the development and progression of each tumor, and for personalized cancer medicine, optimize the preventive and treatment strategies[5,6,56]. For cancer molecular classification, informative biomarkers are needed to be identified to stratify tumors or patients[57-62].

Examples of well-established informative biomarkers include ESR1 (ER-α), PGR and ERBB2 (HER2) expression in breast cancer[63-65], EGFR mutations in lung cancer[66,67], MSI in colorectal cancer[68-70], TMPRSS2-ERG translocation in prostate cancer[71], and TP53, PIK3CA, BRAF and KRAS mutations, and CpG island methylation in multiple cancers[72-74]. Some molecular changes or biomarkers in HCC have also been previously identified. Ojanguren *et al*[75] showed that the positive expression of mutant p53 was related to alcohol abuse (42%) and HBV infection (21%). Park *et al*[76] found that TNF and IL-6 signaling was correlated with obesity-associated HCC development. In the obese patients, insulin and insulin-like growth factors, TNF-α, IL-1 and IL-6, leptin, adipokines, adiponectin, and plasminogen activator inhibitor-1 are significantly associated with the occurrence and development of some cancers, including HCC[77].

### *Molecular epidemiology in HCC*

HCC is also very complex, for example it occurs in about

1%-7% of cirrhotic patients annually, whereas most of the cirrhotic patients do not progress to HCC during their lifetimes[78]. Molecular biomarkers are expected to satisfy this need and resolve the question at the molecular level. To date, molecular epidemiology studies show that a number of molecular risk factors of HCC have been identified, such as numerous genetic polymorphisms reported as host genetic factors[79]. Most of HCC-associated single-nucleotide polymorphisms are identified in genes involved in biological pathways, including oxidative stress (GSTT1, GSTM1), cell cycle (MDM2), immune response (IL10, TNF), DNA damage repair (XPC), growth signaling (EGF), and iron metabolism (HFE) in viral hepatitis- or alcohol-related HCC[80-84]. Recent GWAS identifies the DEPDC5 locus as the risk loci in viral hepatitis-related HCC[85].

Molecular factors associated with etiological agents, for example HBV and HCV could also influence the risk of HCC. It is well known that a high level of serum HBV DNA is indicative of increased risk of HCC. Some studies have demonstrated that HBV genotype is related to the HCC risk[86]. Genomics technology has revealed that HCC should be regarded as a heterogenous group of diseases, not one single disease entity, because each sub-group HCC has different sets of epigenetic and genetic alterations[87]. The heterogeneous molecular features of HCC tumors are associated with the biological behavior, clinical outcome and prognosis[87-91]. Molecular classification is recommended to HCC, and previous studies have identified subsets of HCC tumors characterized by TP53 and CTNNB1 activation mutations, progenitor cell–like features, Met activation, Myc activation, and transforming growth factor-β activation[92-94]. These molecular risk factors of HCC would play important roles in the design and implementation of MPE studies.

### *MPE in HCC*

Epidemiological studies have showed that DM is an established independent risk factor for HCC[12,20-23]; however, how DM affects the development and progression of HCC has not been explained clearly. MPE approaches and studies may be helpful to improve our understanding of the molecular mechanisms of carcinogenesis of HCC. MPE can be used to investigate the relationship between DM and risk of HCC by molecular subtypes. A few MPE studies have been performed for this question, although they were usually under the umbrella of molecular epidemiology. They would be described in the next section in detail. MPE can provide some useful insights for the pathological processes of DM in HCC, although a few challenges must be overcome.

## MPE IN DM AND RISK OF HCC

Currently, based on our knowledge, very few MPE researches are available for DM and risk of HCC[95-97]. For these studies, the original design are not for MPE, and the term of "molecular pathological epidemiology" have not appeared in their articles, but they can be treated as MPE researches, according to the objectives and methods.

One MPE research which was performed in the Japan was designed to determine the relationship between PNPLA3 and JAZF1, and risk of HCC, in patients with non-viral hepatitis and type 2 DM[95]. The objective of this research was to identify genetic determinants associated with T2DM patients who have a high risk of developing HCC by genotyping T2DM susceptibility loci and PNPLA3. This study included 389 T2DM patients, including 59 patients with HCC (DM-HCC) and 330 patients without HCC (DM-non-HCC). Those patients who followed these criteria were included: (1) history of T2DM > 10 years; (2) alcohol intake < 60 g/d; and (3) negative for anti-HCV Ab and HBs-Ag. The authors found that the SNP rs738409 located in PNPLA3 was the greatest risk factor associated with HCC in these diabetic patients. Compared to DM-non-HCC patients, DM-HCC patients had the significantly higher frequency of the PNPLA3 G allele (OR = 2.53, $P = 1.05 \times 10^{-5}$). Moreover, among the 115 DM patients homozygous for the PNPLA3 G allele, HCC patients had the significantly higher frequency of the JAZF1 rs864745 G allele (OR = 3.44, $P = 0.0002$)[95]. They concluded that PNPLA3 and JAZF1 were associated with an increased risk of developing HCC among T2DM patients without viral hepatitis[95].

Another study was designed to evaluate the cytokinome profile, including the serum levels of growth factors, chemokines, cytokines, as well as of other diabetes and cancer biomarkers, in a cohort of patients, including 17 patients with T2DM, 20 patients with chronic hepatitis C infection, 34 patients with HCC, 10 patients with T2DM-HCC, and 20 healthy controls[96]. The results demonstrated that: (1) T2DM-HCC patients had the higher levels of IL-2R, sIL-6Ra, IL-16, IL-18, HGF, β-NGF, CXCL1, CXCL12, ADIPOQ, and IFN-α than those with T2DM or HCC; (2) T2DM-HCC patients had the lower level of LEP than those with T2DM or HCC; (3) T2DM-HCC and only HCC patients had the similar levels of CXCL9, PECAM-1, Prolactin, glucagon, sVEGFR-1 and sVEGFR-2; (4) T2DM-HCC patients had the higher levels of CXCL9, PECAM-1, Prolactin, and glucagon than those with only T2DM; and (5) T2DM-HCC patients had the lower levels of sVEGFR-1 and sVEGFR-2 than those with only T2DM[96]. The major limitation of this study was the very limited number of included patients; however, these molecular changes could be used to design and perform the MPE researches in DM and risk of HCC in future.

Some molecular pathology researches can also be regarded as MPE studies, for example one study which was conducted in the Second Military Medical University, Shanghai, China[97]. The objectives of this study were to determine the effect of p-Ser9-GSK-3β (glycogen synthase kinase-3β) on the prognosis in HCC patients and to explore the interaction between GSK-3β, T2DM and prognosis of HCC. This research included 178 HCC patients after curative partial hepatectomy and showed that expression of P-Ser9-GSK-3β was significantly

higher in tumor tissues than that in their normal counterparts[97]. Moreover, the authors also found that: (1) over-expression of p-Ser9-GSK-3β was associated with T2DM; (2) T2DM and over-expression of p-Ser9-GSK-3β were closely related with each other; and (3) these two variables were independently associated with poor prognosis of HCC[97]. Therefore, p-Ser9-GSK-3β may be regarded as the mediator between T2DM and HCC.

One case report which was published in 2015 was also considered to be related to this field[98]. This report describes a 23-year-old woman with HCC and type 2 DM; and results of histological and immunohistochemical examination showed that this HCC arose in the background of hepatocyte nuclear factor-1α mutated hepatocellular adenomas (H-HCA). However, traditionally, we consider that H-HCA have no minimal malignant potential. For the molecular changes and tumor biomarkers of HCC, the authors found that by immunohistochemical tests, CD34 expression in sinusoidal endothelial cells and expression of glutamine synthetase in tumor cells were increased, whereas exon 3 of CTNNB1 and TERT promoter mutations, and nuclear expression of β-catenin were absent in this patients with HCC and DM. Although such cases are rare, they reinforce the potential of H-HCA for HCC, which may be related to DM[98].

Considering that DM is an independent risk factor for HCC, some efforts have been focused on understanding of the molecular mechanisms of DM in the development and progression of HCC, which may be useful for the design and implementation of MPE studies. For example, one mini-review focused on the impact of TNF-α and IL-6 along with epigenetic regulations[99]. Two approaches are suggested as followed: (1) the first is about the role of TNF-α and IL-6 as inflammatory mediators, from the point of role of apoptosis and inflammation in HCC; and apoptotic regulators can be used for this purpose, such as Bax (bcl-2-like protein 4 encoded by the BAX gene) and Bcl-2 (B-cell lymphoma 2 protein encoded by *BCL2* gene); and (2) the second is about the possible epigenomic reprogramming, from the point of role of epigenetic modification of DNA in HCC. According to these two approaches, apoptotic and inflammatory markers (Bcl2 and Bax), and DNA methylation, hypomethylation or histone modifications can be used as the candidated molecular biomarkers for the understanding of role of DM in HCC[99].

Another review focused on the influence of insulin resistance and hyperinsulinemia of DM in the pathogenesis of hepatocarcinogenesis, and the author summarized that some molecular pathways were involved, for example phosphatase and tensin homolog/P13K/Akt and MAPK kinase[100]. It is well known that different anti-diabetic medications have different influences on the risk of HCC in diabetic patients[23,100]. Metformin has been associated with the decreased risk of HCC in patients diagnosed with DM[23]. The molecular mechanism is deduced that metformin can activate 5-adenosine monophosphate-activated protein kinase (AMPK) and decrease the expression of protein Livin[100]. AMPK can inhibit its downstream target mammalian target of rapamycin, and then inhibit the growth of human cancer cell lines. Livin has been involved in both cell proliferation and survival. Thiazolidinediones seem to inhibit peroxisome proliferator-activated receptor gamma-independent regulation of nucleophosmin and prevent tumor formation[100].

Although these studies are not enough for understanding of molecular mechanisms of DM in the increased risk of HCC, they and the involved molecular biomarkers can be very useful for future MPE researches. I hope that more and more MPE researches are performed exploring the molecular mechanisms as well as novel biomarkers.

## CONCLUSION

DM is an established independent risk factor for HCC; however, how DM affects the occurrence and development of HCC remains as yet unclearly understood. "MPE" is the branch of epidemiology and pathology, and its basis is the molecular classification of tumors. MPE is a multidisciplinary, interdisciplinary and transdisciplinary study field, and molecular pathology plays a central role in this relatively new field. In MPE, investigators examine the relationship between tumor molecular signatures, endogenous and exogenous factors, and development, progression and prognosis of tumors. I believe that this research field can be very helpful to improve our understanding of the pathogenesis, molecular mechanisms, diagnosis, personalized prevention and treatment for DM and risk of HCC in future.

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MINIREVIEWS

# Implication of the intestinal microbiome in complications of cirrhosis

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## Abstract

The intestinal microbiome (IM) is altered in patients with cirrhosis, and emerging literature suggests that this impacts on the development of complications. The PubMed database was searched from January 2000 to May 2015 for studies and review articles on the composition, pathophysiologic effects and therapeutic modulation of the IM in cirrhosis. The following combination of relevant text words and MeSH terms were used, namely intestinal microbiome, microbiota, or dysbiosis, and cirrhosis, encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, hepatopulmonary syndrome, portopulmonary hypertension and hepatocellular carcinoma. The search results were evaluated for pertinence to the subject of IM and cirrhosis, as well as for quality of study design. The IM in cirrhosis is characterized by a decreased proportion of *Bacteroides* and *Lactobacilli*, and an increased proportion of *Enterobacteriaceae* compared to healthy controls. Except for alcoholic cirrhosis, the composition of the IM in cirrhosis is not affected by the etiology of the liver disease. The percentage of *Enterobacteriaceae* increases with worsening liver disease severity and decompensation and is associated with bacteremia, spontaneous bacterial peritonitis and hepatic encephalopathy. Lactulose, rifaximin and Lactobacillus-containing probiotics have been shown to partially reverse the cirrhosis associated enteric dysbiosis, in conjunction with improvement in encephalopathy. The IM is altered in cirrhosis, and this may contribute to the development of complications associated with end-stage liver disease. Therapies such as lactulose, rifaximin and probiotics may, at least partially, reverse the cirrhosis-associated changes in the IM. This, in turn, may prevent or alleviate the severity of complications.

**Key words:** Encephalopathy; Intestinal microbiome; Cirrhosis



**Core tip:** There has recently been an increasing understanding of the importance of the intestinal microbiome (IM) in the physiology of cirrhosis and its complications. Novel sequencing techniques have enabled a better characterization of the bacteria in the IM of patients with cirrhosis, and how this differs from the microbiome in a healthy individual. Additionally, therapeutics for enteric dysbiosis in patients with cirrhosis have been studied, and have shown promise in reducing the morbidity of complications in cirrhosis. In this review, we will critically review the literature on characterization of the IM in cirrhosis, its role in complications, and the evidence for strategies to address enteric dysbiosis.

Bhat M, Arendt BM, Bhat V, Renner EL, Humar A, Allard JP. Implication of the intestinal microbiome in complications of cirrhosis. *World J Hepatol* 2016; 8(27): 1128-1136 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i27/1128.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i27.1128

## INTRODUCTION

Emerging literature has demonstrated that the intestinal microbiome (IM) plays an important role in health and disease. The intestine of a healthy adult harbors 100 trillion intestinal bacteria, and at least 500 different species have been identified with novel molecular biology techniques that allow for sequencing of whole genomes of the IM[1,2]. The healthy adult IM consists principally of *Bacteroides* and *Firmicutes*, which together comprise over 90% of the bacteria present in the colon[3]. The *Bacteroides* are Gram-negative, anaerobic, non-spore-forming bacteria, and especially produce carbohydrate-degrading enzymes, whereas the *Firmicutes* are Gram-positive, anaerobic, spore-forming bacteria, that ferment simple sugars leading to the production of short-chain fatty acids such as butyrate, acetate and propionate[4]. The concentrations of bacteria progressively increase from the proximal to the distal digestive tract, from a maximum of $10^3$ bacteria/mL in the stomach to $10^{12}$ bacteria/mL in the colon[5]. The IM has various functions that affect biochemical, metabolic and physiologic processes both within the intestine and elsewhere in the body[6]. These physiologic functions include the digestion of nutrients, with bacterial disaccharidases transforming unabsorbed dietary sugars to short chain fatty acids (SCFA)[7]. These SCFA can be used as a source of energy by the human body, as they are absorbed through the colon. In addition, butyrate and acetate can be a source of fuel for the enterocytes[8], affect lipid metabolism[9], and have anti-inflammatory effects[10]. Intestinal bacteria can also produce vitamins such as folate and vitamin K[11,12], which are absorbed into the bloodstream[12]. Additionally, the presence of the physiologic IM within the intestinal milieu prevents colonization by pathogenic bacteria and decreases intestinal permeability[8]. Crosstalk between bacteria and enterocytes *via* binding sites and Toll-like receptors help distinguish commensal from pathogenic bacteria[13]. The microbiota then generate an immune response to pathogenic bacteria, and enable oral tolerance by preventing a reaction to dietary protein antigens. Many endogenous bacteria can produce bacteriocins that hinder replication of pathogenic bacterial species[14]. Additionally, commensal microbiota have been shown to promote regulatory T cell function[15] and maturation of natural killer T cells[16]. Finally, many medications including digoxin, opiates, hormones and various antibiotics are transformed into their active forms through intestinal bacterial metabolism. Bacterial deconjugation of glucuronide, sulfate and cysteine conjugates decreases the polarity of drugs, and enables enterohepatic circulation, reabsorption, and prolonged retention[17]. One prime example of a compound whose bacterial metabolism is essential to its activity is sulfasalazine, which is broken down into 5-aminosalicylic acid and sulfapyridine[18]. Additionally, the effects of anticancer immunotherapy can be modulated by the intestinal microbiota. The antitumor effects of CTLA4 blockade were shown to be dependent on specific *Bacteroides* species[19].

Bacterial growth and functions may be affected by several physiologic and anatomic conditions in the GI tract such as peristalsis (may inhibit mucosal attachment of ingested bacteria), the presence of gastric acid and bile (toxic effects), the presence of proteolytic enzymes (degradation of bacteria), the intestinal mucus layer (trapping of bacteria), the ileocecal valve preventing retrograde bacterial translocation[20,21], and secretory IgA inhibiting bacterial proliferation[22]. Changes in small intestinal and colonic motility, gastric acid secretion, bile flow/composition, and the intestinal innate immune response can impact bacterial composition and lead to overgrowth[23]. In addition, external factors such as diet[24], antibiotic use[25] and other environmental factors[26] affect IM composition.

IM composition can also be affected by disease states and vice versa, as shown in various types of autoimmune, metabolic and malignant conditions including colon and gynecologic cancers[3,27-30]. Intestinal microbial dysbiosis, with both qualitative and quantitative changes in bacterial species, has also been associated with the development of obesity[31], diabetes[32], metabolic syndrome[33] and inflammatory bowel disease[34]. In relation to liver disease, recent studies have reported differences in the IM associated with non-alcoholic fatty liver disease (NAFLD)[35,36], alcoholic liver disease[37] and liver cancer[38]. The IM composition in cirrhosis has been shown to be associated with the development of complications, particularly spontaneous bacterial peritonitis and encephalopathy. The goal of this review is to highlight the unique composition of the IM in cirrhosis,

**Table 1 Characterization of Intestinal microbiota across spectrum of liver disease severity**

| Patient population | Changes in IM |
|---|---|
| Healthy patients | Comprised principally of *Bacteroides* and *Firmicutes* (over 90% of IM)[3] |
| Compensated cirrhosis | Higher *Enterobacteriaceae*, *Staphylococcaeae*, and *Enterococcaceae*[53,55,56]<br>Decreased *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiales XIV*, *Bacteroides*, *Faecalibacterium prausnitzii* and *Coprococcus comes*[45,53,55,56] |
| Alcoholic cirrhosis | Higher *Enterobacteriaceae* and endotoxemia compared to other cirrhosis[46] |
| Decompensated cirrhosis | *Enterobacteriaceae* species correlated with increasing MELD score, *Ruminococcaceae* species associated with lower MELD scores[56] |
| Overt hepatic encephalopathy | Higher *Enterobacteriaceae*[57] |
| Hepatorenal syndrome | No established data |
| Hepatocellular carcinoma | No established data |
| Therapeutic strategies and effects on IM | |
| Lactulose | No RCT or prospective studies of microbiome<br>Decreased urea-producing *Klebsiella* and *Proteus* species, increased non-urease-producing lactobacilli[70] |
| Rifaximin | Improved cognitive function due to change in microbiome-metabolome correlation networks, particularly *Enterobacteriaceae* |
| Probiotics | Decreased risk of endotoxemia, TNF-α[74]<br>Enteric dysbiosis reduced, relatively decreased proportion of *Enterobacteriaceae* and *Porphyromonadaceae*[74,75] |
| Fecal microbiota transplantation | Case report data[76]<br>Resolution of hepatic encephalopathy with healthy IM transfer, however IM not characterized |

MELD: Model for end stage liver disease; IM: Intestinal microbiome; RCT: Randomized controlled trial; TNF: Tumor necrosis factor.

its underlying pathophysiology, and its association with clinical manifestations and complications of cirrhosis. Additionally, we will review therapeutic strategies in cirrhosis aimed at restoring a healthy microbiome and at reducing complications.

## RESEARCH AND LITERATURE

The PubMed database was searched from 2000 to May 2015 for studies on the causes, outcomes and modulation of the IM in cirrhosis. The following combination of relevant text words and MeSH terms were used: "IM", microbiome, microbiota, or dysbiosis, and cirrhosis, encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, hepatopulmonary syndrome, portopulmonary hypertension and Hepatocellular carcinoma (HCC). Our search included both original and review articles as well as letters to the editor. We (Mamatha Bhat and Venkat Bhat) obtained 369 abstracts, manually searching the abstracts for pertinence to the subject of IM and cirrhosis. This resulted in 46 entries that provided information on the etiology, pathophysiology, characterization of the IM, and management of enteric dysbiosis in cirrhosis. Table 1 summarizes the results of our systematic review.

## ENTERIC DYSBIOSIS IN LIVER DISEASE

Emerging literature suggests that the IM is not only altered in liver disease of various etiologies, but that this dysbiosis may play an etiopathogenetic role. For example, dysbiosis may contribute to NAFLD[35,39] by contributing to enhanced hepatic fat accumulation[39]. In a cross-sectional study, patients with non-alcoholic steatohepatitis (NASH) had a significantly lower percentage of *Bacteroides* in their stool[35] compared to patients with simple steatosis and healthy controls, although a cause-effect relationship could not be established. Mechanisms engendered by microbial genes, such as an increase in appetite signaling, energy extraction from the diet, and expression of lipogenic genes likely contribute to enhanced hepatic fat accumulation[31,40]. In addition, hepatic inflammation and fibrosis in patients with NASH are thought to occur due to bacterial translocation of intestine-derived microbial products (including endotoxin) and activation of Toll-like receptor (TLR) signaling[41,42]. This results in stimulation of hepatic stellate cell activity, with subsequent liver fibrogenesis[43]. The role of bacterial lipopolysaccharide (endotoxin) in fibrogenesis has been confirmed in mouse models, where TLR4 knockout significantly decreased expression of markers for liver fibrosis such as collagen, α-smooth muscle actin, procollagen-I, transforming growth factor-β1 and matrix metalloproteinase-2[44]. It is thought that enteric dysbiosis in the context of liver disease of any etiology contributes through the above mechanism to liver disease progression.

## CHARACTERIZATION OF IM IN CIRRHOSIS

### *Evidence of bacterial overgrowth in cirrhosis*

Patients with cirrhosis have both qualitative and quantitative changes in their gut microbiota[45-47]. Small intestinal bacterial overgrowth, defined as $>10^5$ CFU/mL and/or the presence of colonic bacteria in upper jejunal aspirate, is present in 48% to 73% of cirrhotic patients[48,49]. Impaired small intestinal motility[50], decreased bile flow[51], and dysregulated secretion of immunoglobulin A[51] and antimicrobial molecules[52] all contribute to small intestinal bacterial overgrowth in patients with cirrhosis (Figure 1).

### *IM composition in cirrhosis*

In addition to this increased bacterial burden, taxonomic

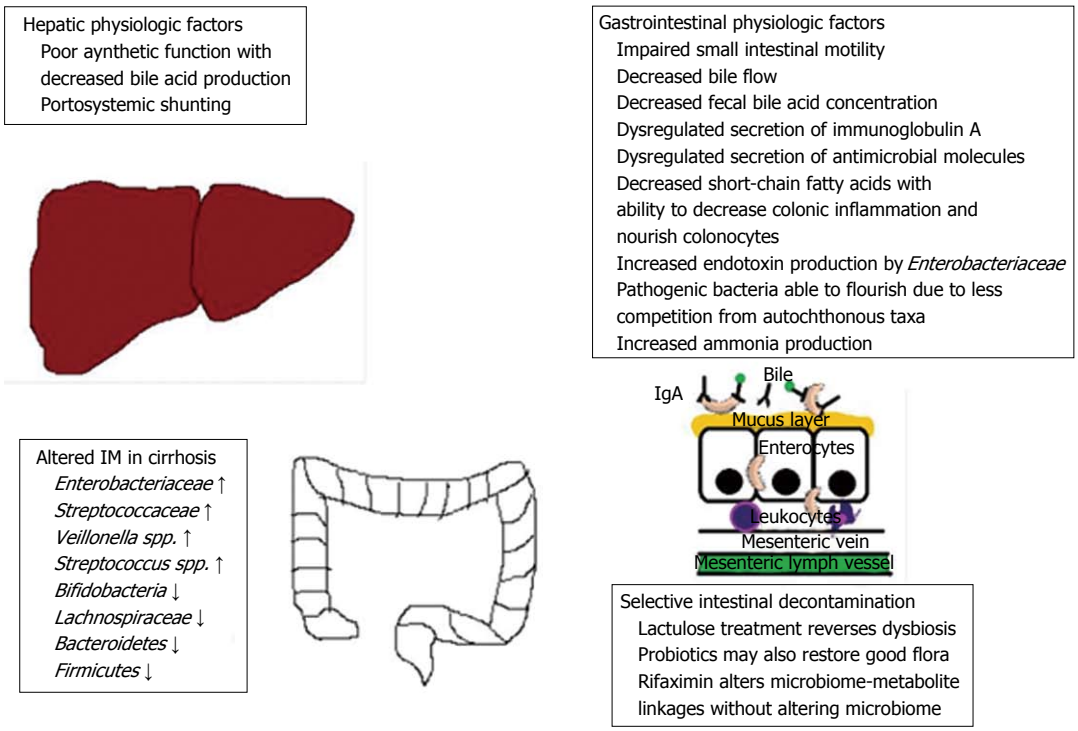


**Figure 1 Factors influencing intestinal microbiome composition in cirrgosis.** IM: Intestinal microbiome.

differences in the fecal microbial communities have been demonstrated[51,53-56]. Patients with cirrhosis commonly have decreased proportions of beneficial, autochthonous taxa, such as *Lachnospiraceae, Ruminococcaceae* and *Clostridiales XIV*. There is a relative overgrowth of potentially pathogenic bacteria such as *Enterobacteriaceae, Staphylococcaeae*, and *Enterococcaceae*, whose abundance correlates with disease progression and endotoxemia[53,55,56]. A recent study comparing the microbiome in cirrhosis *vs* healthy controls revealed that the beneficial *Bacteroides* genus was significantly decreased in patients with cirrhosis[24]. Additional bacterial species that enrich the health and diversity of the microbiome, such as *Faecalibacterium prausnitzii* (anti-inflammatory properties) and *Coprococcus comes* (butyrate production) were found to occupy a relatively lower proportion of the microbiome in cirrhosis[41]. Most studies have shown that the etiology of cirrhosis does not affect taxonomic composition, with similar fecal microbial communities across the spectrum of liver disease etiologies[51,57]. Recently however, the pattern of dysbiosis has been reported to be slightly different in alcoholic cirrhosis, with higher *Enterobacteriaceae* and a higher proportion of patients with endotoxemia compared to cirrhotic patients of non-alcoholic etiologies. This held true after adjusting for severity of disease (MELD score) and abstinence[42].

### *Mechanisms associated with dysbiosis in cirrhosis*

The beneficial, autochthonous taxa of the IM generate SCFA that sustain colonocytes and decrease inflammation, in addition to anti-bacterial peptides that help prevent colonization by pathogenic taxa and reinforce the intestinal barrier[58]. The decreased presence of certain benign bacteria is thought to be due to decreased bile acid production in cirrhosis. This environment allows pathogenic bacteria to thrive and outgrow the "beneficial" species[6]. The combination of a decreased, "leaky" intestinal barrier with increased endotoxin production by pathogenic taxa such as the *Enterobacteriaceae* can lead to endotoxemia[41].

### *IM and severity of cirrhosis*

The IM composition appears to vary with the severity of cirrhosis and the presence of complications. The increased presence of the *Streptococaceae* taxon has been correlated with worsening Child-Pugh score, whereas the *Lachnospiraceae* taxon was associated with less severe disease[53]. However, the Child-Pugh score includes hepatic encephalopathy as a component, and encephalopathy itself is associated with a distinct IM as described further below. Later studies of the IM in cirrhotics have therefore employed the MELD score, in order to allow for simple correlation of the IM with severity of liver dysfunction. *Enterobacteriaceae* species have been reported to be associated with higher MELD scores, whereas the *Ruminococcaceae* species have been associated with lower MELD scores[56]. A study of the IM in patients with advanced liver disease revealed that decreased abundance of *Bifidobacterium* and increased abundance of *Enterococcus* were associated with increasing liver dysfunction[59]. The term "cirrhosis dysbiosis ratio" was coined to describe the ratio of autochthonous taxa (taxa that are benign and usually present in the gut such as *Ruminococcaceae*, *Lachnospiraceae*, and *Clostridiales*) to non-autochthonous ones (*Enterobacteriaceae* and *Bacteroidaceae*). This ratio was highest

among healthy individuals as expected, and decreased with worsening MELD score and degree of hepatic decompensation[57]. Those with compensated cirrhosis had a cirrhosis-dysbiosis ratio of 0.89, whereas those with decompensated cirrhosis had a ratio of 0.66, and patients hospitalized for cirrhosis related complications had a ratio of 0.32 ($P < 0.0001$). An increase in the relative abundance of pathogenic bacteria was associated with the development of complications such as hepatic encephalopathy. Liver disease stability over months was associated with a stable cirrhosis-dysbiosis ratio[57].

Interestingly, salivary dysbiosis is concomitantly present with enteric dysbiosis, with a relative increased abundance of *Enterobacteriaceae* and decrease in autochthonous species[60]. Dysbiosis of the salivary microbiome was particularly pronounced in patients requiring 90-d liver-related hospitalizations. Thus, the salivary microbiome may serve as a substitute for the IM, and would be an easier sample to obtain.

Patients with cirrhosis show changes in both serum and fecal bile acids, which results from decreased liver synthetic function, altered enterohepatic circulation and altered IM composition. Overgrowth of *Enterobacteriaceae* leads to impaired conversion of primary to secondary bile acids[61]. This results in a decreased ratio of secondary to primary bile acids, along with a reduced overall fecal bile acid concentration, which correlate with increasing severity of liver disease. These findings are accompanied by a concomitant increase in serum bile acids[61].

## IMPACT OF MICROBIOME ON COMPLICATIONS OF CIRRHOSIS

### *IM and sepsis*

Complications of end-stage liver disease, such as spontaneous bacterial peritonitis and hepatic encephalopathy, have been linked to pathological bacterial translocation. The translocation of bacteria or their products (such as muramyl-dipeptides, lipopolysaccharides (endotoxin), peptidoglycans and bacterial DNA) from the intestine to the mesenteric lymph nodes is a normal physiological process that bolsters host immunity[62]. Pathological bacterial translocation occurs due to an increase in the rate or degree of translocation. This is the case in cirrhosis, given the leaky intestinal barrier and relatively immunodeficient state[49]. The bacteria causing SBP are mostly gram-negative bacilli such as *Escherichia coli* and other members of the *Enteriobacteriaceae* family (*Proteus, Klebsiella* and *Enterobacter*), which are present in higher abundance in the gut microbiota of cirrhotic patients[51,53,55,56]. Migration of these bacteria to the peritoneal cavity or systemic circulation results in peritonitis and bacteremia, respectively. Pathological bacterial translocation triggers inflammation and the hyperdynamic circulation of cirrhosis that contributes to portal hypertension. These in turn result in serious systemic infections with up to 38% mortality[6,63]. Therefore, translocation of bacteria from an altered IM represents an important determinant of mortality in cirrhotic patients.

### *IM and hepatic encephalopathy*

The IM contributes to development of hepatic encephalopathy through ammoniagenesis and an endotoxin-driven inflammatory response. Additional compounds produced by the microbiota, such as mercaptans, phenols, short- and medium-chain fatty acids and benzodiazepine-like compounds, potentially contribute as well[2]. In a metagenomic study of the microbiome in cirrhosis, Qin *et al*[64] performed in-depth assessment of functions of the microbiome enriched in liver cirrhosis. In cirrhosis, bacterial genes involved in the assimilation or dissimilation of nitrate to or from ammonia, denitrification, gamma-aminobutyric acid biosynthesis, and amino acid transport were highly represented[64]. Additionally, manganese-related transport system modules were enriched in the IM of patients with cirrhosis[64]. This may be associated with manganese accumulation within the basal ganglia of cirrhotic patients, which is thought to contribute to hepatic encephalopathy[65]. A patient discrimination index was developed based on a group of 15 bacterial species, and it was highly accurate as a biomarker for cirrhosis. In addition to the enteric dysbiosis described above, altered intestinal permeability results in translocation of bacteria and their products, which has an important effect on the progression of cirrhosis[66].

### *IM and hepatocellular carcinoma*

HCC is another complication of cirrhosis whose development may be influenced by the altered IM in the cirrhotic patient[67], although there is no concrete evidence as yet. It is well known that chronic inflammation can foster the initiation and progression of malignancies. Translocation of intestinal bacteria can lead to hepatic inflammation, with release of key inflammatory mediators such as NF-κB and TLR4. Downregulation of the NF-κB signaling pathway *in vivo* (by ablating the protein that activates this transcription factor) was shown to sequentially induce NAFLD, fibrosis and finally HCC[68]. TLR4 and the IM contributed to tumor progression in an HCC mouse model, and have therefore been proposed as chemopreventive targets[69]. This study suggests that the IM may have adverse effects on hepatic stellate cell function, activating the release of inflammatory mediators that promote HCC development.

## MODULATION OF IM IN CIRRHOSIS

### *Lactulose*

The longstanding practice of treating hepatic encephalopathy with lactulose not only decreases ammonia absorption, but also results in modulation of the IM with decreased ammonia production[70]. Lactulose acidifies the colonic pH, which renders the environment hostile to the urease-producing *Klebsiella* and *Proteus* species. Conversely, the intestinal lumen becomes friendlier to non-urease-producing lactobacilli and bifidobacteria.

The end-result of these changes in the microbiome is decreased ammonia production[71].

### Antibiotics

Antibiotics such as neomycin, metronidazole and ciprofloxacin have been used in the past to treat hepatic encephalopathy, although the IM was never characterized in this context. More recently, rifaximin has been offered to patients with lactulose-resistant hepatic encephalopathy. In a prospective, open-label study, 20 cirrhotic patients with minimal hepatic encephalopathy were treated with rifaximin 550 mg twice daily for 8 wk[72]. The IM, serum metabolome, and cognitive function were assessed before and after rifaximin treatment in all 20 patients. Although a significant improvement in cognitive function and endotoxemia was seen, the composition of the IM was not distinctly different. Rather, there was a shift from pathogenic to beneficial metabolite linkages around pathogenic bacterial species (*Enterobacteriaceae* and *Porphyromonadaceae*). Therefore, although the IM composition itself was not altered, the metabolic profile produced by the pathogenic species was more beneficial. On the other hand, the correlation networks around the autochthonous bacteria (looking at the interactions between the microbiome and metabolome) remained the same. This study therefore illustrated how rifaximin could alter intestinal microbial linkages with metabolites, without any significant effect on microbial composition or abundance *per se*[72].

### Probiotics

The effect of probiotic therapy on the IM in cirrhotic patients has also been studied[73]. One appealing benefit of probiotics is their excellent safety profile. A phase I, 8-wk, randomized controlled trial of the probiotic Lactobacillus GG in 30 patients with minimal hepatic encephalopathy revealed that it was safe and well-tolerated, while decreasing the risk of endotoxemia and lowering TNF-$\alpha$ in the serum, plasma and liver[74]. Enteric dysbiosis was reduced, with a relatively decreased proportion of *Enterobacteriaceae* and *Porphyromonadaceae* (both associated with worse disease in cirrhosis). Conversely, the abundance of autochthonous species like *Lachnospiraceae, Ruminococcaceae, and Clostridiales XIV* increased. There was no change in the *Lactobacillaceae* abundance, and it was hypothesized that this species either promoted colonization by beneficial microbiota or enhanced intestinal epithelial function and the immune system, thereby displacing pathogens[74]. Additionally, changes in metabolites related to amino acid, vitamin and secondary bile acid were found. Cognition however was not improved, although this trial was a phase I study without the statistical power to determine this outcome[72,74].

A second randomized, double-blind, placebo-controlled trial of VSL#3 daily for 6 mo assessed the probiotic's efficacy in preventing recurrent encephalopathy, reducing severity of liver disease and reducing hospitalizations[75]. There was a tendency towards decreased episodes of recurrent encephalopathy (primary outcome), with 34.8% in the probiotic group *vs* 51.6% in the placebo group ($P$ = 0.12). In addition, there was a significantly reduced risk of hospitalization, as well as improved Child-Pugh and MELD scores with daily use of VSL#3.

### Fecal microbiota transplantation

This is a potentially interesting approach to addressing enteric dysbiosis, although the only evidence to date is a single case report where healthy gut microbiota transfer was used to treat hepatic encephalopathy[76]. This was recently described in a case report of a patient with Grade 1-2 encephalopathy not responsive to lactulose, and unable to afford rifaximin. Fecal microbiota from a healthy stool donor was transplanted into the patient by colonoscopy and by retention enemas weekly over a 5-wk period. The patient's alertness, as well as his performance on measures of encephalopathy (inhibitory control test and Stroop test) significantly improved and normalized. This case demonstrates that fecal microbiota transplantation is a plausible strategy in treating mild encephalopathy by correcting enteric dysbiosis, although further larger-scale studies are required.

In summary, the IM is significantly altered in cirrhosis, with a decrease in beneficial, autochthonous bacterial species such as *Bacteroides*, and an increase in pathogenic bacteria such as the *Enterobacteriaceae*. Except for alcoholic liver disease, IM composition appears to be similar across etiologies of hepatic cirrhosis. The dysregulated IM likely is associated with and may contribute to the development of complications of end-stage liver disease, including hyperdynamic circulation, portal hypertension, bacteremia, spontaneous bacterial peritonitis, and encephalopathy. The role of the IM in the development of hepatorenal syndrome and HCC is suspected, but not yet elucidated. Treatment with lactulose, antibiotics, and probiotics may be effective in preventing or improving these complications by targeting the enteric dysbiosis.

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ORIGINAL ARTICLE

Clinical Trials Study

# Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease

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## Abstract

***AIM***

To investigate the independent effects of 6-mo of dietary energy restriction or exercise training on whole-body and hepatic fat oxidation of patients with non-alcoholic fatty liver disease (NAFLD).

***METHODS***

Participants were randomised into either circuit exercise training (EX; $n$ = 13; 3 h/wk without changes in dietary habits), or dietary energy restriction (ER) without changes in structured physical activity (ER; $n$ = 8). Respiratory quotient (RQ) and whole-body fat oxidation rates ($Fat_{ox}$) were determined by indirect calorimetry under basal, insulin-stimulated and exercise conditions. Severity of disease and steatosis was determined by liver histology; hepatic $Fat_{ox}$ was estimated from plasma β-hydroxybutyrate concentrations; cardiorespiratory fitness was expressed as $\dot{V}O_{2peak}$. Complete-case analysis was performed (EX: $n$ = 10; ER: $n$ = 6).

***RESULTS***

Hepatic steatosis and NAFLD activity score decreased with ER but not with EX. β-hydroxybutyrate concentrations increased significantly in response to ER (0.08 ± 0.02 mmol/L *vs* 0.12 ± 0.04 mmol/L, $P$ = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 mmol/L *vs* 0.11 ± 0.07 mmol/L, $P$ = 0.39). Basal RQ decreased ($P$ = 0.05) in response to EX, while this change was not significant after ER ($P$ = 0.38). $VO_{2peak}$ ($P$ < 0.001) and maximal $Fat_{ox}$ during aerobic exercise ($P$ = 0.03) improved with EX but not with ER ($P$ > 0.05). The increase in β-hydroxybutyrate concentrations was correlated with the reduction in hepatic steatosis ($r$ = -0.56, $P$ = 0.04).

***CONCLUSION***

ER and EX lead to specific benefits on fat metabolism of patients with NAFLD. Increased hepatic $Fat_{ox}$ in response to ER could be one mechanism through which the ER group achieved reduction in steatosis.


**Key words:** Non-alcoholic steatohepatitis; Steatosis; Fat and carbohydrate oxidation; Exercise; Fitness; Beta-hydroxybutyrate; Ketone bodies; Fatty acid oxidation



**Core tip:** We investigated hepatic fat oxidation and whole-body substrate oxidation under basal, insulin-stimulated and exercise conditions before and after 6 mo of circuit exercise training (EX) or dietary energy restriction (ER) in patients with non-alcoholic fatty liver disease. ER increased β-hydroxybutyrate concentrations (a marker of hepatic fat oxidation) and reduced severity of steatosis, but did not change substrate oxidation rates during acute exercise. EX improved substrate oxidation under basal, insulin-stimulated and exercise conditions, but not β-hydroxybutyrate concentrations and severity of disease. Increase in β-hydroxybutyrate was associated with decrease in hepatic steatosis and this could be one mechanism through which the ER group achieved reduction in steatosis.

Croci I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O'Moore-Sullivan TM, Prins JB, Macdonald GA, Hickman IJ. Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease. *World J Hepatol* 2016; 8(27): 1137-1148 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i27/1137.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i27.1137

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in industrialised countries and its prevalence is increasing globally[1]. The term NAFLD describes a range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis that occur in the absence of hazardous alcohol consumption. NAFLD is linked with obesity, visceral adiposity, physical inactivity, insulin resistance[2], and genetic predisposition[3]. Intrahepatic triglycerides (TGs) (steatosis) accumulate when the sum of *de novo* hepatic fatty acid synthesis rate and hepatic fatty acid uptake rate is greater than those of TG export and hepatic fat oxidation[4]. In a recent cross-sectional study we have shown that overweight patients with NAFLD do not adequately adapt fuel oxidation to fuel availability, with reduced fat oxidation rates ($Fat_{ox}$) in resting and fasting conditions, a reduced suppression of $Fat_{ox}$ after insulin stimulation and a lower increase in $Fat_{ox}$ during exercise compared to lean controls[5]. Further, we observed that patients with NAFLD had reduced hepatic $Fat_{ox}$, as measured by plasma β-hydroxybutyrate, when compared to lean controls.

Lifestyle interventions consisting of diet (improved diet quality with or without energy restriction) or diet in conjunction with exercise training are currently the most commonly advocated therapies for NAFLD management[6-8]. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body $Fat_{ox}$. Hallsworth *et al*[9] showed that 8 wk of resistance training without weight loss did not change substrate oxidation rates in the basal state (resting and fasting) but increased $Fat_{ox}$ during aerobic exercise. However, substrate oxidation during exercise was assessed at a single intensity and at the same absolute intensity pre and post intervention (50% of the pre-intervention $\dot{V}O_{2peak}$). Therefore, assessment of maximal rate of $Fat_{ox}$ (MFO) and the intensity at which it occurs ($Fat_{max}$) was not possible, and participants likely were assessed at a lower relative intensity post-intervention (due to improved $\dot{V}O_{2peak}$). Gaining a deeper understanding of substrate metabolism during exercise is of interest because the full body metabolic demands are higher and potential alterations not observable at rest may become apparent.

The effect of different treatment options for NAFLD on

hepatic $Fat_{ox}$ is also unclear. In response to dietary energy restriction (ER), little information is available. A study in which 18 patients with NAFLD underwent 2 wk of dietary ER reported increased plasma β-hydroxybutyrate concentrations (indicating increased hepatic $Fat_{ox}$), and this was correlated with reduction in steatosis[10]. This is in agreement with findings in animal models showing that an increase in hepatic $Fat_{ox}$ leads to a reduction in hepatic steatosis[11,12]. However, whether a similar response is seen in response to a longer dietary intervention, with the assessment being performed in energy balance (as opposed to energy deficit), needs to be established. Furthermore, the effect of an exercise training program on plasma β-hydroxybutyrate concentrations is unknown[13]. Understanding the independent effect of energy restriction and exercise training on whole-body $Fat_{ox}$ and hepatic $Fat_{ox}$ in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management.

Improvement in cardiorespiratory fitness (CRF) is a key endpoint in exercise training interventions. Cross-sectional evidence shows that lower levels of physical activity and CRF correlate with more severe hepatic injury on histology and greater steatosis[14-17]. However, the relationship between change in CRF measured with a graded exercise test and change in steatosis (measured quantitatively) has not been explored longitudinally in NAFLD[18,19]. Investigating the associations between changes in markers of CRF, substrate oxidation, and histological, metabolic and biochemical features of NAFLD in response to exercise can help understand the mechanisms through which exercise may benefit features of NAFLD.

This study aimed to investigate changes in hepatic $Fat_{ox}$ and in whole-body substrate oxidation rates under basal, insulin-stimulated and exercise conditions, in patients with NAFLD who completed either 6 mo of dietary energy restriction or circuit exercise training. The second aim was to assess whether changes in CRF, whole-body fat and hepatic $Fat_{ox}$ were associated with changes in hepatic steatosis.

## MATERIALS AND METHODS

### Participants

Overweight patients with NAFLD (diagnosed on liver biopsy) participated in the study ($n$ = 21). Exclusion criteria included: Type 2 diabetes, cirrhosis, decompensated liver disease, presence of other causes of liver disease, and daily ethanol consumption > 20 g in females or > 40 g in males. The study was approved by the local Human Research Ethics Committees (Princess Alexandra Hospital and University of Queensland, Australia). All participants provided informed written consent. The randomised controlled clinical trial was registered with the Australian and New Zealand Clinical Trials Registry (http://www.anzctr.org.au). The registration identification number is ACTRN12612001087842.

### General design

Participants were randomised into either a dietary energy restriction intervention (ER; $n$ = 8) or an exercise training intervention (EX; $n$ = 13). A consort diagram describing the flow of patients through the randomised controlled trial is presented in Figure 1. Outcome measures were assessed prior to randomisation (pre-intervention) and after 6 mo of intervention. At both time-points participants undertook three testing sessions within a 7-d period. Patients had stable body weight for at least 2 wk before the post intervention testing.

During the first testing session, body composition was assessed by dual-energy X-ray absorptiometry. The second session involved a hyperinsulinaemic-euglycaemic clamp with indirect calorimetry measurements to assess substrate oxidation rates under basal and insulin-stimulated conditions. This session also involved clinical assessments, including blood pressure and anthropometry. During the third testing session, indirect calorimetry measurement was performed during a graded exercise test on an ergocycle to determine substrate oxidation rate and CRF (as measured by $\dot{V}O_{2peak}$). The second and third sessions were conducted in the morning after an overnight fast. Both ER and EX groups were instructed not to change exercise and physical activity patterns throughout the intervention and this was monitored with accelerometers at three time points during the intervention.

The primary outcomes of the trial were hepatic steatosis and IR and have been published elsewhere[20]. The present manuscript focuses on secondary outcome measures including plasma β-hydroxybutyrate concentrations, and whole-body $Fat_{ox}$ under basal, insulin-stimulated and exercise conditions. The flow of participants for the present analysis is presented in the Consort Diagram in Figure 1. Complete-case analysis, including 10 EX and 6 ER participants, was performed.

### Exercise training intervention

EX, as previously detailed[20], involved 3 sessions per week of circuit exercise training during 6 mo without dietary restriction. The aim was to improve CRF, muscle strength and body composition without significant body weight loss. EX was selected based on preliminary research conducted in our laboratory[21].

Training intensity was fixed at 50% of 1-RM for the entire duration of the training program; 1-RM was reassessed monthly to account for strength adaptations. The training volume was progressively increased from one circuit (12 min) in week 1 to five circuits (60 min) in week 11; and then remained constant at five circuits from week 11 until the end of the intervention. Each circuit comprised 12 light resistance exercises covering the major muscle groups. The training program consisted of alternating 30 s exercise intervals and 30 s rest periods. Pneumatic resistance training machines were employed (Ab Hur Oy, Kokkola, Finland). All training sessions were supervised by an exercise physiologist.

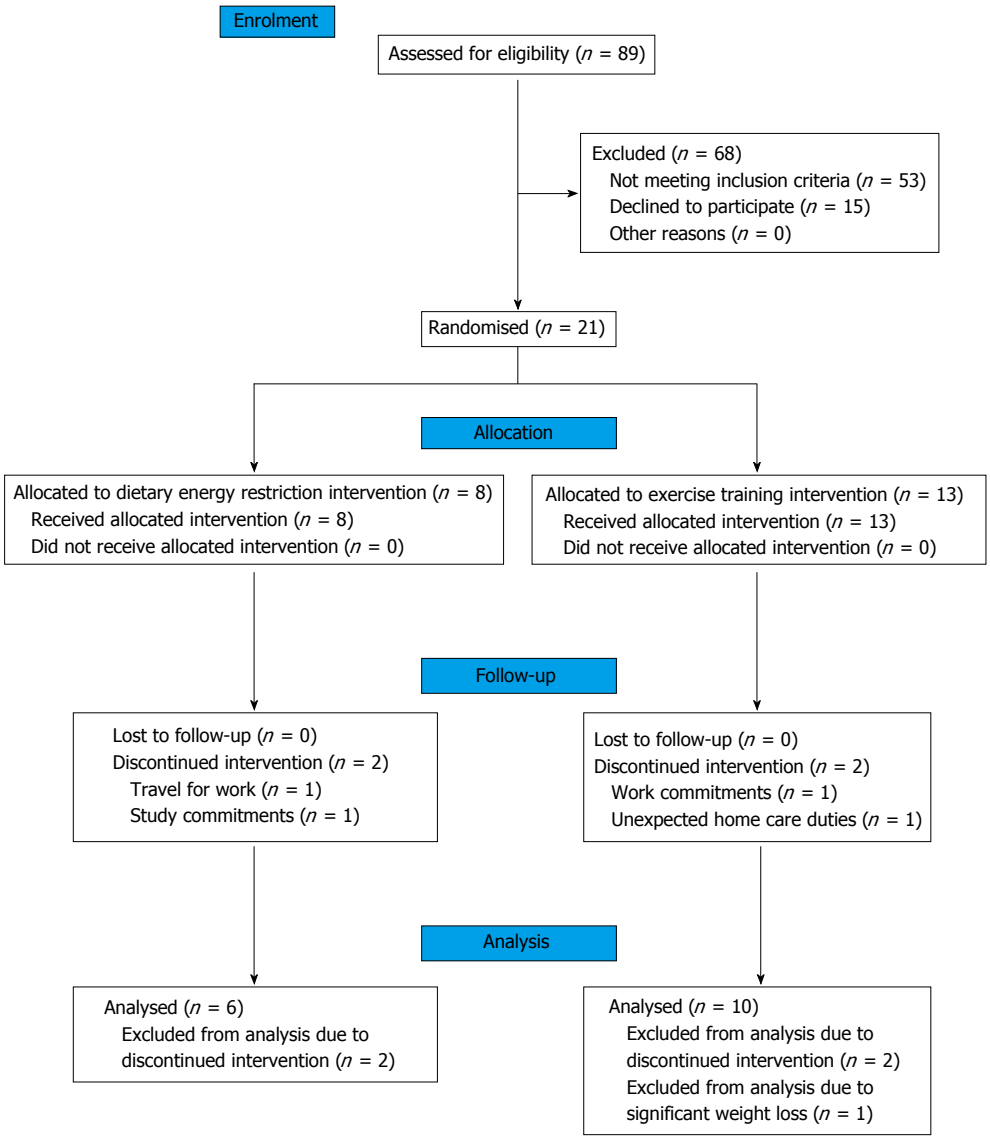


**Figure 1 Consort diagram describing the flow of patients through the randomised controlled trial.**

### *Energy restriction*

ER involved a weight loss program under the guidance of a dietitian. Patients attended weekly face-to-face appointments for 16 wk and were provided with an individualised dietary prescription with the aim of 5%-10% of body weight loss within 16 wk. This was followed by an 8-wk period aimed at body weight maintenance, with fortnightly reviews with the dietitian. The target macronutrient composition was 40% carbohydrate, 20% protein and 40% fat (< 10% saturated fat). Recommendations included choosing foods that are low in saturated fats; avoiding micronutrient-poor/energy-dense food options; avoiding added sugar; and aiming for regular meal patterns. Weekly weight and waist measures, and 24-h diet recalls encouraged adherence and self-monitoring.

### *Histological analysis of liver biopsy*

Liver biopsy specimens were analysed as previously detailed[5,20]. The severity of liver injury was determined with the NAFLD activity score (NAS)[22] and the criteria described by Brunt[23]. Using conventional histologic criteria[24], a diagnosis of NASH or steatosis alone was made.

### *Body composition*

Body composition assessments including determination of fat-free mass (FFM) and fat mass by dual-energy X-ray absorptiometry. Subcutaneous abdominal fat and visceral abdominal fat were assessed by computed tomography as previously described[25].

### *Insulin sensitivity*

Insulin sensitivity was assessed with the hyperinsulinemic-euglycemic clamp technique[26], as we previously detailed[20]. Briefly, primed insulin was infused at a rate of 1 mU/kg per minute throughout the procedure (2 h), and a 25% glucose solution was infused at a variable rate to maintain euglycemia[26]. The glucose infusion rate in the steady state of the hyperinsulinemic-euglycemic clamp

(M-value) corresponded to the whole-body glucose disposal rate.

### Biochemical analysis

Biochemical analyses were performed as previously described[5,20]. Plasma β-hydroxybutyrate concentrations, an index of hepatic ketogenesis[27-30], were measured with an enzymatic assay (Stanbio, Boerne, TX, CV 2.2%).

### Exercise testing

Maximal aerobic power and substrate utilization were assessed with a graded exercise test on an ergocycle. Testing comprised a sub-maximal phase to determine $Fat_{ox}$ and $CHO_{ox}$ at multiple intensities (with workload increments occurring every 5 min), and a maximal phase to assess peak oxygen consumption ($\dot{V}O_{2peak}$) (increments every min). The testing protocol adopted has been described in detail in a previous publication[5].

### Indirect calorimetry measurements

Indirect calorimetry measurements (TrueOne 2400 Metabolic Measurement System, Parvo Medics, UT) were conducted in three physiological states (basal, insulin-stimulated and exercise). Whole-body $Fat_{ox}$ and $CHO_{ox}$ were calculated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible[31]. The methodological approach adopted has been previously described in detail[5].

$Fat_{ox}$ rates during exercise were estimated from respiratory gazes averaged over the last minute of each exercise stage. Then, the stage at which MFO was achieved was determined, and the corresponding intensity was identified ($Fat_{max}$)[32]. ΔRQ represented the RQ change from basal to hyperinsulineamic state (RQ in the insulin-stimulated condition minus basal RQ).

Testing sessions involving indirect calorimetry measurements were conducted in the morning after a 10-12 h overnight fast and under standardised conditions[5]. Standardisation of pre-test conditions was in line with previous studies[32-40].

### Daily physical activity

Daily physical activity was quantified with RT3 accelerometers Activity Monitor, 2003, Stayhealthy, Incorporated, Monrovia, CA, United States) worn for 7 consecutive days at 0, 3 and 6 mo, as previously described[20].

### Statistical analysis

A secondary analysis of outcomes from a larger clinical trial was performed. Independent *t*-tests were used to compare the pre-intervention (baseline) characteristics between groups (ER *vs* EX). Paired Student *t*-tests were used to compare within group outcome measures pre and post intervention. Wilcoxon matched-pair signed rank test was used if samples were not normally distributed. Correlation analyses were performed using Pearson's correlation coefficient or Spearman's non-parametric rank correlation coefficient. As outlined in the consort diagram (Figure 1), complete-case analysis was performed. Complete-case analysis was deemed more suitable than intention to treat analysis given that the aim of this study was to study mechanisms of benefit of the two interventions. Statistical analysis was performed with SPSS 17.0 (SPSS, Chicago, IL, United States) and Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, United States). Data are expressed as mean ± SD or median and range. For all statistical analyses, the level of significance was set at $P < 0.05$. Statistical methods used in this study were reviewed a biostatistician.

## RESULTS

### Characteristics of study groups

Two patients from each arm ($n = 4$) did not complete the study due to time constraints. One participant ($n = 1$) from the EX group was excluded from analysis due to significant weight loss at 6 mo (-13.3% body weight, which cannot be achieved with the type and volume of exercise prescribed as part of this exercise intervention). Data analysis (complete-case analysis) was thus performed on 10 participants from the EX and 6 participants from the ER groups (see the Consort Diagram presented in Figure 1). There were no significant differences between pre-intervention patients' characteristics of completers and non-completers. Compliance with both interventions was good. The ER group achieved an average weight loss of 9.7% ± 4.6%, and the EX group attendance to the exercise sessions was greater than 90% with no significant weight loss. As per protocol, usual daily time spent on low, moderate and high intensity physical activity did not change in either group ($P > 0.05$). ER and EX interventions were well tolerated by participants with no adverse events reported.

Characteristics of the EX and ER groups are presented in Table 1. At baseline, the prevalence of NASH was not different between ER and EX groups (67% *vs* 80%, $P = 0.64$). Primary results of the randomised controlled trial are reported elsewhere[20]. Briefly, in the ER group steatosis and the NAS decreased significantly, while in the EX group neither steatosis nor NAS decreased significantly. Skeletal muscle insulin resistance (M-value) improved significantly in response to EX, while it did not improve in patients from the ER group.

### Substrate oxidation under basal conditions

Total energy expenditure in resting and fasted conditions (basal) did not significantly change in response to both interventions ($P > 0.05$). However, with the EX intervention the relative contribution of fat and CHO to energy expenditure changed: The RQ and the $CHO_{ox}$ decreased (by 30%, $P = 0.02$), while $Fat_{ox}$ tended to increase (Table 2). With the ER intervention, the same direction of change as for EX was seen, however statistical significance was not reached. In the whole-group, the pre-post intervention change in basal RQ was not associated with the pre-post intervention changes in steatosis ($r = 0.05$, $P = 0.88$) or

**Table 1 Characteristics of the study groups at baseline (pre-intervention) and after 6 mo of energy restriction or exercise training (post-intervention)**

| | Energy restriction ($n$ = 6) | | Exercise training ($n$ = 10) | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Age (yr) | 45.5 ± 13.5 | | 51.8 ± 6.7 | |
| Gender (M:F) | 3:3 | | 7:3 | |
| BMI (kg/m$^2$) | 33.5 ± 9.0 | 30.0 ± 7.0[a] | 31.2 ± 3.2 | 30.8 ± 3.5 |
| Fat-mass (%) | 38 ± 9 | 35 ± 11[b] | 36 ± 7 | 33 ± 6[a] |
| Fat-free mass (kg) | 54.1 ± 12.3 | 51.3 ± 11.8 | 63.1 ± 14.3 | 64.4 ± 14.2[a] |
| Waist (cm) | 106 ± 16 | 90 ± 13 | 110 ± 14 | 105 ± 13[a] |
| Systolic blood pressure (mmHg) | 126 ± 13 | 118 ± 13[a] | 139 ± 19 | 137 ± 18 |
| Subcutaneous adipose tissue (cm$^2$) | 358 ± 282 | 268 ± 202[b] | 322 ± 116 | 298 ± 117[a] |
| Visceral adipose tissue (cm$^2$) | 202 ± 110 | 203 ± 56[b] | 182 ± 67 | 117 ± 36[a] |
| Diastolic blood pressure (mmHg) | 83 ± 8 | 75 ± 12 | 88 ± 11 | 83 ± 10 |
| Triglycerides (mmol/L) | 1.6 ± 0.8 | 1.1 ± 0.4 | 2.0 ± 1.3 | 2.0 ± 0.2 |
| HDL cholesterol (mmol/L) | 0.9 ± 0.2 | 1.0 ± 0.3 | 1.0 ± 0.2 | 1.1 ± 0.2[a] |
| LDL cholesterol (mmol/L) | 3.5 ± 0.8 | 3.0 ± 0.6 | 3.2 ± 1.1 | 3.1 ± 1.0 |
| VLDL cholesterol (mmol/L) | 0.7 ± 0.3 | 0.5 ± 0.2[b] | 0.9 ± 0.6 | 0.7 ± 0.5 |
| Free fatty acids (mmol/L) | 0.59 ± 0.15 | 0.63 ± 0.23 | 0.59 ± 0.17 | 0.62 ± 0.25 |
| Glucose (mmol/L) | 5.2 ± 0.3 | 5.0 ± 0.7 | 5.5 ± 0.5 | 5.3 ± 0.4 |
| Insulin (mU/L) | 18 ± 18 | 10 ± 5 | 24 ± 23 | 12 ± 10 |
| M-value (mg/kgFFM per minute) | 4.2 ± 1.4 | 5.2 ± 1.5 | 4.0 ± 0.9 | 5.2 ± 1.6[a] |
| hsCPR (mg/L) | 4.9 ± 3.7 | 2.0 ± 1.6 | 3.9 ± 3.6 | 1.5 ± 1.3 |
| Alanine aminotransferase (U/L) | 80 ± 65 | 55 ± 55 | 54 ± 19 | 49 ± 28 |
| Aspartate aminotransferase (U/L) | 40 ± 22 | 28 ± 16 | 38 ± 11 | 39 ± 22 |

Complete-case analysis performed. [a]$P$ < 0.05, within group difference in reponse to the intervention; [b]$P$ value < 0.10, within group trend in reponse to the intervention. Pre-intervention there was no difference between energy restriction and exercise groups in any of the parameters presented ($P$ > 0.05). M:F: Male: Female; BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; hsCRP: High sensitivity C reactive protein.

NAS ($P$ = 0.35).

### β-hydroxybutyrate concentrations

As shown in Figure 2, basal plasma β-hydroxybutyrate concentrations, increased significantly in response to ER (0.08 ± 0.02 mmol/L *vs* 0.12 ± 0.04 mmol/L, $P$ = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 mmol/L *vs* 0.11 ± 0.07 mmol/L, $P$ = 0.39). This result (unchanged β-hydroxybutyrate concentrations in response to EX) was confirmed also when the analysis was performed excluding the outlier (0.09 ± 0.03 mmol/L *vs* 0.09 ± 0.03 mmol/L, $P$ = 0.87) (Figure 2). In the combined cohort including participants from both groups, there was a negative association between pre-post intervention changes in β-hydroxybutyrate and in hepatic steatosis ($r$ = -0.56, $P$ = 0.04) (Figure 3). This relationship persisted after controlling for changes in body weight ($r$ = -0.67, $P$ = 0.02) and percentage body weight ($r$ = -0.56, $P$ = 0.05).

### Substrate oxidation under insulin-stimulated conditions

Hyperinsulinaemic concentrations were reached by both groups at both times points (ER, 79.0 ± 31.5 mU/L *vs* 80.0 ± 21.5 mU/L; EX, 83.0 ± 0.5 mU/L *vs* 78.1 ± 18.0 mU/L; all $P$ > 0.05). The effect of the two interventions on substrate oxidation in insulin-stimulated conditions is presented in Table 3. Post-intervention, the EX group tended to increase the insulin-stimulated suppression of $Fat_{ox}$ compared with pre-intervention (-0.24 ± 0.36 mg/kgFFM per minute *vs* -0.55 ± 0.35 mg/kgFFM per minute, $P$ = 0.06). The ER group displayed a similar response, however statistical significance was not reached. In the pooled group, the pre-post intervention increase in ΔRQ (change in RQ from the basal to the insulin-stimulated state) was not correlated with the change in the severity of steatosis ($r$ = 0.28, $P$ = 0.28) or NAS ($P$ = 0.31).

### Substrate oxidation during exercise

$\dot{V}O_{2peak}$ and MFO improved significantly (by 18% and 71%, respectively) in response to EX but did not change in the ER group (Table 4 and Figure 4). $Fat_{max}$ increased by 72% in response to EX when expressed in absolute terms (45 ± 20 *vs* 76 ± 46 Watts, $P$ = 0.03), whereas it remained unchanged after both interventions when expressed in relative terms (%$\dot{V}O_{2peak}$). Within the EX group, the increase in $\dot{V}O_{2peak}$ (mL/kgFFM per minute) was correlated with the increase in ΔRQ ($r$ = 0.73, $P$ = 0.02) and the reduction in systolic blood pressure ($r$ = -0.81, $P$ = 0.01). The improvement in $\dot{V}O_{2peak}$ was not related with the change in steatosis ($r$ = 0.14, $P$ = 0.73), NAS ($P$ = 0.40) or basal RQ ($r$ = -0.18, $P$ = 0.62). Similarly, the change in MFO was not related to changes in hepatic steatosis ($r$ = 0.03, $P$ = 0.91), or changes in NAS ($P$ = 0.63).

## DISCUSSION

ER and EX are standard interventions for the management of obesity and related comorbidities, including NAFLD. ER

**Table 2 Resting substrate metabolism pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention) in patients with non-alcoholic fatty liver disease**

| | Energy restriction ($n$ = 6) | | | Exercise ($n$ = 10) | | |
|---|---|---|---|---|---|---|
| | Pre | Post | *P* | Pre | Post | *P* |
| Respiratory quotient | 0.82 ± 0.04 | 0.80 ± 0.04 | 0.38 | 0.84 ± 0.06 | 0.81 ± 0.06 | 0.05 |
| $Fat_{ox}$ (mg/kgFFM per minute) | 1.18 ± 0.25 | 1.46 ± 0.33 | 0.17 | 1.15 ± 0.54 | 1.35 ± 0.48 | 0.08 |
| $CHO_{ox}$ (mg/kgFFM per minute) | 2.33 ± 0.69 | 1.72 ± 0.83 | 0.19 | 2.70 ± 1.24 | 1.90 ± 1.17 | 0.02 |

Complete-case analysis performed. $Fat_{ox}$: Fat oxidation rates; $CHO_{ox}$: Carbohydrate oxidation rates; FFM: Fat-free mass.

**Table 3 Change in substrate metabolism from basal (resting and fasting) to insulin-stimulation conditions pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention)**

| | Energy restriction ($n$ = 6) | | | Exercise ($n$ = 10) | | |
|---|---|---|---|---|---|---|
| | Pre | Post | *P* | Pre | Post | *P* |
| Δ Respiratory quotient | 0.05 ± 0.05 | 0.08 ± 0.05 | 0.58 | 0.04 ± 0.02 | 0.07 ± 0.05 | 0.11 |
| Δ $Fat_{ox}$ (mg/kgFFM per minute) | -0.29 ± 0.46 | -0.56 ± 0.32 | 0.31 | -0.24 ± 0.36 | -0.55 ± 0.35 | 0.06 |
| Δ $CHO_{ox}$ (mg/kgFFM per minute) | 0.92 ± 0.98 | 1.41 ± 0.98 | 0.46 | 0.54 ± 0.85 | 1.02 ± 0.93 | 0.18 |

Complete-case analysis performed. $Fat_{ox}$: Fat oxidation rates; $CHO_{ox}$: Carbohydrate oxidation rates; FFM: Fat-free mass; Δ: Change from basal to insulin-stimulated condition.

**Table 4 Maximal aerobic power and substrate oxidation during exercise pre-intervention, and after 6 mo of energy restriction or exercise treatment (post-intervention)**

| | Energy restriction ($n$ = 6) | | | Exercise ($n$ = 10) | | |
|---|---|---|---|---|---|---|
| | Pre | Post | *P* | Pre | Post | *P* |
| $\dot{V}O_{2peak}$ (mL/kg per minute) | 20.4 ± 5.1 | 20.7 ± 6.4 | 0.73 | 23.9 ± 6.4 | 28.3 ± 6.3 | < 0.001 |
| $\dot{V}O_{2peak}$ (mL/kgFFM per minute) | 32.5 ± 5.0 | 31.0 ± 5.4 | 0.31 | 39.2 ± 8.4 | 43.6 ± 7.4 | 0.004 |
| Workload at $\dot{V}O_{2peak}$ (W) | 121 ± 53 | 121 ± 57 | 0.94 | 176 ± 78 | 224 ± 81 | < 0.001 |
| MFO (g/min) | 0.14 ± 0.13 | 0.06 ± 0.04 | 0.17 | 0.17 ± 0.09 | 0.29 ± 0.14 | 0.03 |
| MFO (mg/kgFFM per minute) | 2.5 ± 1.7 | 1.2 ± 0.7 | 0.18 | 2.8 ± 1.5 | 4.4 ± 1.9 | 0.04 |
| Workload at MFO (W) | 44.8 ± 16.5 | 41.3 ± 13.4 | 0.43 | 44.7 ± 19.5 | 76.3 ± 46.0 | 0.03 |
| $Fat_{max}$ (%$\dot{V}O_{2peak}$) | 48.7 ± 14.7 | 47.9 ± 8.8 | 0.62 | 45.2 ± 12.3 | 47.0 ± 7.2 | 0.94 |

Complete-case analysis performed. $\dot{V}O_{2peak}$: Peak oxygen uptake; MFO: Maximal fat oxidation; W: Watts; $Fat_{max}$: Exercise intensity eliciting maximal fat oxidation; FFM: Fat-free mass.

induced weight loss, reduced hepatic steatosis, increased β-hydroxybutyrate concentrations (a marker of hepatic $Fat_{ox}$) but did not lead to changes in substrate oxidation rates tested during an acute exercise session. EX lead to improvements in CRF and in substrate oxidation rates under basal, insulin stimulated and exercise conditions. However, this dose of circuit exercise did not lead to improvements in hepatic $Fat_{ox}$ or hepatic steatosis. In the combined cohort, the reduction in hepatic steatosis was associated with increased β-hydroxybutyrate concentrations.

A novel finding from this study was that ER and EX interventions had different effects on β-hydroxybutyrate concentrations in patients with NAFLD. In response to ER, the increase in β-hydroxybutyrate (product of the oxidation pathway) was accompanied by the trend for a decrease in the very low-density lipoprotein (product of the esterification pathway), despite no change in free fatty acids concentrations. These are favourable changes given that pre-intervention patients with NAFLD showed lower β-hydroxybutyrate and higher very low-density lipoprotein compared to healthy controls[5]. These changes may suggest that the ER intervention lead to a change in hepatic fatty acid partitioning, with free fatty acids being more directed towards oxidation than towards esterification[41]. Increase in hepatic $Fat_{ox}$ could be a mechanism through which the ER group achieved reduction in steatosis. Accordingly, it was shown in animal models that interventions that increase hepatic $Fat_{ox}$ lead to a reduction in hepatic steatosis[11,12].

In contrast, β-hydroxybutyrate concentrations remained unaltered in response to EX. This observation is valuable because, as highlighted in a recent review, no information is available on the chronic effects of exercise training on β-hydroxybutyrate concentrations[13]. Results from the present study do not confirm findings from rodent models, which showed that chronic exercise training increased hepatic $Fat_{ox}$[42] and that the shift from an active to a sedentary lifestyle reduced hepatic $Fat_{ox}$[43]. Future studies assessing the effects of different training prescriptions (volume, intensity, frequency, duration) and the optimal type of exercise (aerobic *vs* circuit *vs* resistance) on hepatic lipid metabolism are warranted. Inclusion of genetic and molecular parameters in future investigations might provide insights on the mechanisms responsible for the inter-individual variability observed in

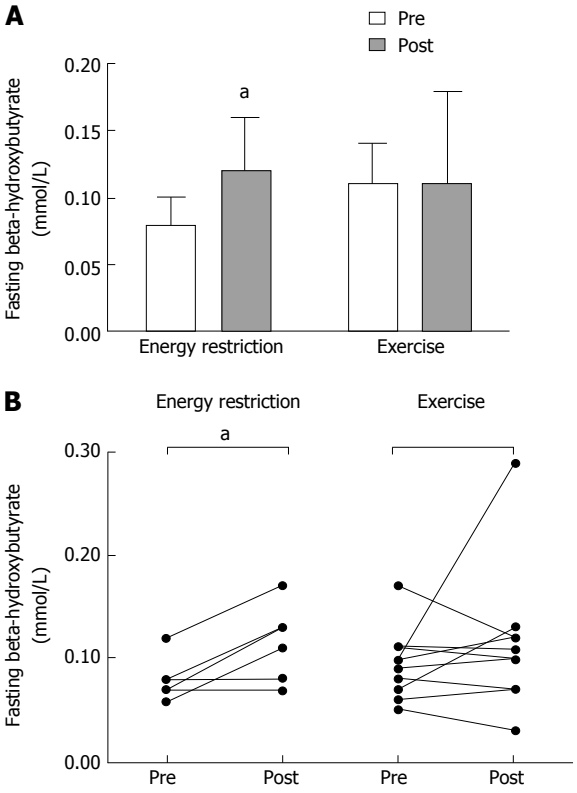


**Figure 2 Basal β-hydroxybutyrate concentrations before and after 6 mo of energy restriction (*n* = 6) or exercise training (*n* = 10).** A: Average responses; B: Individual responses. [a]*P* < 0.05 between pre and post treatment.

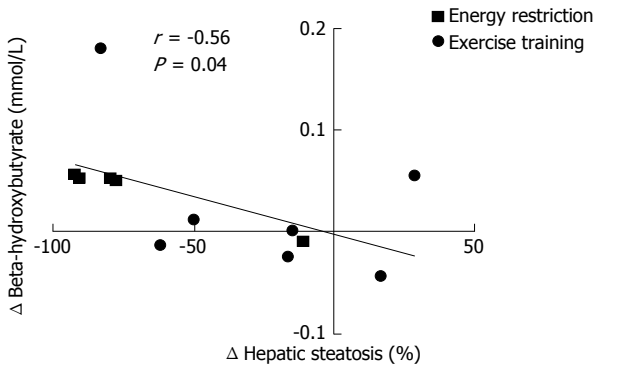


**Figure 3 Relationship between change in β-hydroxybutyrate concentrations and relative change in hepatic steatosis in response to 6 mo of energy restriction or exercise training (*n* = 13).** This relationship remained significant after controlling for changes in body weight (*r* = -0.67, *P* = 0.02).

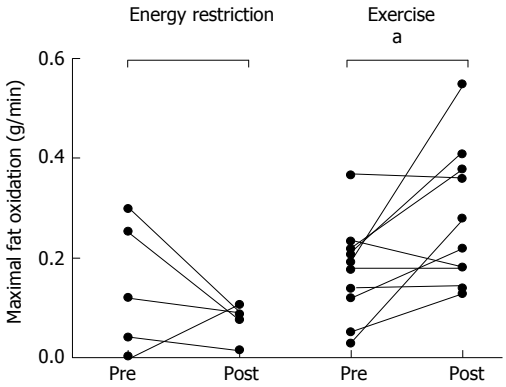


**Figure 4 Maximal fat oxidation before and after six months of energy restriction (*n* = 6) or exercise training (*n* = 10); individual data.** [a]*P* < 0.05 between pre and post intervention.

response to the treatments.

The effect of the two interventions on MFO was different: It markedly increased in response to EX, while it remained unchanged in response to ER. The improvement in MFO in the exercise group could be attributable to increased mitochondrial content, increased oxidative capacity and improved transport of free fatty acids across muscle and mitochondrial membranes[44-47]. Such changes likely were not achieved in response to ER[48]. To our knowledge, this was the first study comparing the effect of two types of lifestyle intervention (*i.e.*, ER and EX) on MFO in patients with NAFLD. It was also the first study to assess $Fat_{max}$ and MFO in response to circuit exercise training. The improvement observed in MFO was consistent with previous studies conducted in other populations: Higher whole-body $Fat_{ox}$ during exercise was observed in response to a moderate intensity aerobic training program conducted in obese males[49], and in response to high-intensity aerobic training[50] or resistance exercise training[51] programs conducted in healthy individuals. Overall, the improvement in MFO in response to EX and lack of change in response to ER are in agreement with findings from a recent cross-sectional study showing that substrate oxidation rates during exercise are correlated with CRF but not with body weight or percentage body fat[52].

Another observation from the present study was that EX improved whole-body substrate oxidation rates in resting and insulin-stimulated conditions (greater $Fat_{ox}$ in basal conditions and greater increase in $CHO_{ox}$ in response to insulin stimulation). The increased basal whole-body $Fat_{ox}$ observed in response to EX treatment is in agreement with studies conducted in obese patients[53,54]. On the other hand, no change was observed by the only other study which investigated whole-body fat oxidation in response to exercise training in NAFLD. The different outcome compared to the present study could be explained by the shorter duration of the intervention (8 wk) and the different baseline characteristics of the study population (less severe NAFLD)[9]. In response to ER, there appeared to be a change towards a greater proportion of basal energy expenditure derived from $Fat_{ox}$, however statistical significance was not achieved due to the small sample size. These results are in line with other dietary interventions involving high-fat diets with carbohydrate restriction[55-58]. Increase in whole-body $Fat_{ox}$ after treatment is of relevance in this patient population because in a recent cross-sectional study[5] we showed that whole-body $Fat_{ox}$ is reduced in patients with NAFLD compared to healthy controls, and that this alteration was associated with the degree of steatosis.

This study comprehensively investigated the independent effects of ER and EX, the cornerstones of lifestyle treatment, on fat and carbohydrate oxidation assessed in

different physiological conditions including basal, insulin stimulation, and exercise. This forms an ideal framework to study changes in whole-body energy homeostasis and elucidate mechanisms of change in response to a therapy. Assessment of severity of liver disease, insulin resistance and body composition were conducted using gold standard techniques. A further strength was that the EX program was supervised by an exercise physiologist and was the longest exercise training intervention performed in NAFLD to date.

The randomised controlled trial was powered for detecting within group changes in primary outcome measures (hepatic steatosis and M-value), meaning that type 2 error for other outcome measures cannot be excluded. However, this did not interfere with the interpretation of key results of the present manuscript (*i.e.,* β-hydroxybutyrate concentrations and $Fat_{ox}$ during exercise) given that statistically significant differences were still observed. The sample size was relatively small but it was comparable to those from similar studies conducted in NAFLD to date[9,59]. Further, a very specific population was studied: Patients were non-diabetic with histologically proven NAFLD and a large proportion (> 75%) of those patients had NASH, which represents an important distinction because patients with NASH are more likely to progress to end stage liver disease[60]. Finally, it must be acknowledged that β-hydroxybutyrate concentrations, while being a commonly used marker of hepatic $Fat_{ox}$[41], do not represent a direct measure of hepatic $Fat_{ox}$. Future studies assessing the effect of lifestyle intervention in NAFLD on rates of hepatic fatty acid uptake, oxidation, and storage using a newly validated method combining $^{11}$C-palmitate imaging by positron emission tomography with compartmental modelling[61], would be of interest. Studies including assessment of redox metabolism and gene expression are also warranted.

Based on the length of intervention and type of exercise training provided, the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should remain unchanged to recommend a combination of both ER and exercise training given that these interventions provide complementary benefits. EX is particularly beneficial for improving skeletal muscle fat metabolism and CRF, while ER provided greater benefits on hepatic fat metabolism[6]. Future research is required to investigate the impact of different doses and types of exercise programs on the severity of disease as well as on hepatic and whole-body substrate metabolism. Dose and type of exercise are likely to be crucial factors impacting on the clinical benefits of an exercise intervention[62,63]. To date, the beneficial effects of exercise training on NAFLD have been mostly seen in response to aerobic training[59,64-69] or with an aerobic component[9]. It is possible that aerobic exercise training has a greater impact on hepatic steatosis and hepatic $Fat_{ox}$ than other training regimes because during aerobic exercise substrate availability is more closely matched with substrate oxidation and energy deficit is greater than during other training regimes.

In conclusion, this study showed ER and EX, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. ER induced weight loss, increased β-hydroxybutyrate concentrations in basal condition, reduced severity of steatosis and severity of disease, but did not lead to changes in substrate oxidation rates during an acute exercise session. EX without weight loss, lead to improvements in substrate oxidation under basal, insulin-stimulated and exercise conditions. However, this dose of circuit exercise training was not sufficient for improvements in β-hydroxybutyrate and severity of liver disease. Increased hepatic $Fat_{ox}$ in response to ER could be one of the mechanisms through which the ER group achieved reduction in steatosis.

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## COMMENTS

### *Background*

Lifestyle interventions consisting of diet or diet in conjunction with exercise training are currently the most commonly advocated therapies for non-alcoholic fatty liver disease (NAFLD) management. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body and hepatic fat oxidation in NAFLD.

### *Research frontiers*

Understanding the independent effect of diet and exercise on whole-body and hepatic fat oxidation in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management. Exercise training as a treatment option to reduce the burden of NAFLD is an emerging field of research.

### *Innovations and breakthroughs*

This study showed diet and exercise, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. Dietary energy restriction provided greater hepatic benefits, while exercise training provided greater peripheral (whole-body) improvements.

### *Applications*

Based on the length of intervention and type of exercise program provided (6 mo of circuit exercise training), the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should continue to recommend a combination of both diet and exercise given that these interventions provide complementary benefits.

### *Terminology*

β-hydroxybutyrate is a ketone body produced uniquely by the liver, therefore plasma concentrations of β-hydroxybutyrate are used as an index of hepatic fat oxidation or hepatic ketogenesis.

### *Peer-review*

Authors comment adequately the only problem of this study, which is the short number of individuals who completed the study. Results are interesting and the

study is well conducted.

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ORIGINAL ARTICLE

Prospective Study

# Ohio solid organ transplantation consortium criteria for liver transplantation in patients with alcoholic liver disease

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## Abstract

***AIM***

To evaluate risk of recidivism on a case-by-case basis.

***METHODS***

From our center's liver transplant program, we selected patients with alcoholic liver disease who were listed for transplant based on Ohio Solid Organ Transplantation Consortium (OSOTC) exception criteria. They were considered to have either a low or medium risk of recidivism, and had at least one or three or more months of abstinence, respectively. They were matched based on gender, age, and Model for End-Stage Liver Disease (MELD) score to controls with alcohol-induced cirrhosis from Organ Procurement and Transplant Network data.

***RESULTS***

Thirty six patients with alcoholic liver disease were approved for listing based on OSOTC exception criteria and were matched to 72 controls. Nineteen patients

(53%) with a median [Inter-quartile range (IQR)] MELD score of 24 (13) received transplant and were followed for a median of 3.4 years. They were matched to 38 controls with a median (IQR) MELD score of 25 (9). At one and five years, cumulative survival rates (± standard error) were 90% ± 7% and 92% ± 5% and 73% ± 12% and 77% ± 8% in patients and controls, respectively (Log-rank test, $P$ = 0.837). Four (21%) patients resumed drinking by last follow-up visit.

***CONCLUSION***

Compared to traditional criteria for assessment of risk of recidivism, a careful selection process with more flexibility to evaluate eligibility on a case-by-case basis can lead to similar survival rates after transplantation.

**Key words:** Alcohol-induced disorders; Alcoholic liver cirrhosis; Mortality; Survival; Liver transplantation



**Core tip:** For the first time, we report the rates of liver transplant and survival for patients with alcohol-induced cirrhosis who were deemed eligible for liver transplant and listed based on approval under the Ohio Solid Organ Transplantation Consortium medically urgent exception criteria. These criteria allow patients with low to medium risk of recidivism, to receive a liver transplant after only one to three months of abstinence. We showed that transplant rate and short and long term survival after transplant is comparable between these patients and United States general population of patients with alcohol-induced cirrhosis who received liver transplant.

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## INTRODUCTION

Cirrhosis due to alcoholic liver disease is an important cause of morbidity and mortality both globally and in the United States. Globally, in 2010 cirrhosis due to alcoholic liver disease led to more than 493000 deaths[1]. In United States in 2011 liver cirrhosis was responsible for 34860 deaths, 48% of which were related to alcohol consumption[2]. Among patients with cirrhosis due to alcoholic liver disease mortality rates vary based on presence or absence of complications of cirrhosis but it is generally high with a one-year mortality ranging from 17% to 64% and five-year mortalities ranging from 58% to 85%[3].

Liver transplantation imparts great survival benefit to appropriately selected patients with advanced and de-compensated cirrhosis due to alcohol consumption, which is comparable to survival benefit of transplant in other types of chronic liver disease[4,5]. The definition of "appropriately selected patients" in this context remains controversial[5], with the most important factor being minimum duration of abstinence. Conventionally, most liver transplant programs in United States require patients to be abstinent for at least 6 mo and participate in an alcohol rehabilitation program to be considered for transplant[6,7]; while it is known that delayed referral for transplant and longer waiting times, even for a few months, will significantly decrease the probability of patient's survival in the pre-transplant period[8]. There are data suggesting that careful evaluation of patients for transplant on an individual basis instead of using general and inflexible enrollment rules might lead to favorable outcomes in highly selected patients with alcoholic liver disease[9,10]. The state of Ohio Solid Organ Transplantation Consortium (OSOTC) provides such a mechanism for case-by-case evaluation based on clinical guidelines for medically urgent patients with cirrhosis due to alcoholic liver disease. Based on factors such as estimated risk of recidivism, severity of their alcohol use history and previous attempts to remain sober, social support, insight into alcohol use, and willingness of the patient to comply with OSOTC regulations, these patients can be approved as an exception and listed for transplant after only one to three months of abstinence.

The aim of this study was to determine the effect of using OSOTC transplant eligibility criteria on patients' survival compared with conventional criteria for assessment of risk of recidivism, in patient with cirrhosis due to alcoholic liver disease.

## MATERIALS AND METHODS

### *Study population and data collection*

The study protocol was approved by the Cleveland Clinic Institutional Review Board. Since 2009 we selected patients with alcoholic cirrhosis for consideration of liver transplantation based on OSOTC exception criteria. No donor organs were obtained from executed prisoners or other institutionalized persons. As defined below these are medically urgent patients with low to medium risk of recidivism, who were approved for a medically urgent exception to be transplanted either during the time they were completing alcohol treatment, or some completed treatment after their transplant.

Transplant rates were compared between these patients and matched patients with alcoholic cirrhosis from Organ Procurement and Transplant Network (OPTN) data records who had complete data to calculate their Model for End-Stage Liver Disease (MELD) score[11,12] at the time of listing. To compare survival after transplant we used the OPTN patients with alcoholic cirrhosis who had complete data to calculate their MELD score at the time of transplant as well as follow-up data on survival after transplant. Patients from OPTN dataset

**Table 1 Ohio Solid Organ Transplantation Consortium medically urgent except criteria**

| | Ohio Solid Organ Transplantation Consortium Criteria |
|---|---|
| Low-risk | 1 mo confirmed abstinence, a signed contract and commitment to begin a rehabilitation program and finish it either before or after transplant<br>No previous failure with substance rehabilitation; never been told that substance was affecting health; and good social support |
| Medium-risk | Three month confirmed abstinence, a signed contract and commitment to begin a rehabilitation program and/or finish it either before or after transplant<br>One or more failures with rehabilitation; and minimal support system |
| High-risk | Two or more failures to remain abstinent despite medical complication<br>Refusal to sign contract<br>Minimal to poor social support<br>Must complete standard criteria treatment plan, not eligible for an exception |
| Other barriers | No insight into their alcohol use consequences<br>No recognition that alcohol caused their liver failure<br>Refusal to start treatment<br>No sober support network |

were matched to our patients randomly and according to the following predetermined variables: 10-year age category, gender, and MELD score category same as the case patient's category (< 10, 10-19, 20-29, 30-39, $\geqslant$ 40)[13]. MELD score was calculated as (9.57 × log creatinine mg/dL) + (3.78 × log bilirubin mg/dL) + (11.20 × log international normalized ratio) + 6.43. All laboratory values which were less than 1 were set to 1 and serum creatinine for patients with values of more than 4 or on dialysis was set to 4 in order to calculate MELD score. The MELD score was truncated at 40 for patients with a MELD score of more than 40 (http://optn.transplant.hrsa.gov/resources/MeldPeldCalculator.asp?index=98).

### *OSOTC chemical disorder criteria*

OSOTC follows standard criteria for patients in need of liver transplant who are diagnosed with substance use disorder at the time of evaluation (Table 1). This includes patients with alcohol-induced cirrhosis who are diagnosed with alcohol use disorder. The standard criteria includes demonstrating abstinence for at least 12 mo before listing, or at least three months of abstinence plus three months of current participation in an active recovery program and negative random toxicology screens prior to listing confirmed by collateral information (http://www.osotc.org/resources/chemical-dependency-criteria/). In addition, patients must show insight into substance use and understanding of the effects of substance use on their health.

OSOTC also provides exception criteria for medically urgent patients who have not been abstinent for 12 mo and are too ill to complete the recovery program participation conditions in the standard criteria. These exception criteria apply to patients with MELD score of more than 22 (calculated or eligible for exception). According to OSOTC exception criteria, and after signing a contract and showing commitment to rehabilitation, patients at low risk of recidivism - defined as no previous failure with substance rehabilitation, never having been told that substance was affecting health, and good social support - can be listed for transplant after one month of abstinence. Patients at medium risk of recidivism - defined as one or more failures with rehabilitation, and minimal support system - can be listed after a minimum of three months of abstinence, and after signing a contract and showing commitment to rehabilitation. All patients' records are reviewed by OSOTC chemical disorder committee representatives and discussed in a committee conference call, in addition to our liver transplant patient selection committee, in order to decide approval or not of these exception criteria. Patients at high risk for recidivism - defined as two or more failures to remain abstinent despite medical complications, refusal to sign a contract, and minimal or poor social support - do not qualify for OSOTC exception criteria.

### *Statistical analysis*

All analysis was done with Stata Data Analysis and Statistical Software (version 11.2 SE, StataCrop LP). Variables are reported as number (percentage) or median (IQR). Survival probabilities are reported as percentage ± SE. Categorical variables are compared between patients and controls with $\chi^2$ test. Waiting time was defined as the period from the day an individual is listed for liver transplant to the day the transplant is done. Waiting time was compared between cases and controls with a Cox proportional hazards model containing patient group as the only independent variable to predict waiting time (*i.e.,* time to liver transplant). Follow-up time after transplant was defined as the period from the day an individual receives a liver transplant until death or the last follow-up visit. Data for patients who remained alive by the end of the follow-up period was censored at the time of last follow-up visit. Survival probabilities were estimated with Kaplan-Meier method and were compared between groups with Log-Rank test. All *P*-values are two-sided.

## RESULTS

### *Patients' selection*

Between 2009 and 2013, 326 patients with alcoholic liver disease were evaluated for liver transplant at the Cleveland Clinic, of whom 279 (85%) patients were considered high-risk for recidivism or alcohol relapse based on the OSOTC criteria (Figure 1). These high-risk patients underwent the standard chemical dependency requirements defined above before being considered eligible for liver transplant. Forty-seven (15%) patients were considered by our social workers and liver transplant committee at the Cleveland Clinic to be at medium or low-risk for recidivism or alcohol relapse based on the OSOTC criteria, but only 36 (13%) patients were approved by the consortium.

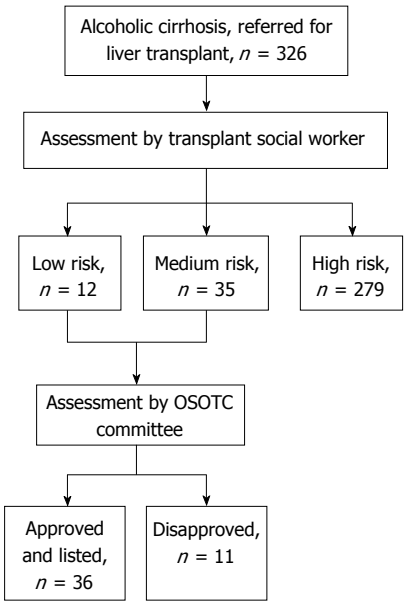


**Figure 1 Selection of patients with alcoholic liver disease for liver transplantation based on Ohio Solid Organ Transplantation Consortium Criteria.** OSOTC: Ohio Solid Organ Transplantation Consortium.

### *Baseline characteristics*

Thirty-six patients with alcoholic cirrhosis were approved for liver transplant at the Cleveland Clinic based on OSOTC exception criteria. They were matched based on age, gender, and MELD score category to a random sample of 72 controls from OPTN database with alcohol-induced cirrhosis that underwent liver transplant following conventionally used criteria of alcohol rehabilitation. Table 2 represents the baseline characteristics of patients and control groups. Sixty four percent of patients and controls were male. At the time of listing five patients had a MELD score of 10-19 (14%), 18 had a MELD score of 20-29 (50%), 12 had a MELD score of 30-39 (33%), and one patient (3%) had a MELD score of 40 or more. These were individually matched to controls with the same MELD score category, leading to a median (IQR) MELD score of 27 (11) among patients and 24 (11) among controls (Table 1). At the time of liver transplant one patient had a MELD score of less than 10 (5%), two had MELD scores of 10-19 (11%), 11 had MELD scores of 20-29 (58%), four had MELD scores of 30-39 (21%), and one patient had a MELD score of 40 or more (5%). Again, these patients were individually matched to controls with the same MELD score category leading to a median (IQR) MELD score of 24 (13) among patients and 25 (9) among controls.

### *Liver transplantation*

Nineteen out of 36 (53%) patients received a liver transplant and 17 dropped off the transplant list. The most common cause of drop-off transplant list was infection and the vast majority of dropped off patients died ($n$ = 15, 88%). The transplant drop-off rate was not different for controls of whom 41 (57%) received a transplant ($P$-value = 0.681). Patients in the OSOTC group received their liver after a median waiting time of 19 d after listing, and controls received their transplant after a median 21 d of waiting time ($P$-value = 0.648). Although the majority of both patients and controls received their transplant in less than 2 mo (Table 3), 10% of controls had to wait more than five months while all patients received their transplants before the five months mark.

**Table 2 Characteristics of study patients and controls**

| | Patients | Controls |
|---|---|---|
| At listing | | |
| *n* | 36 | 72 |
| Age, median (IQR) | 58 (14) | 60 (11) |
| Male, *n* (%) | 23 (64) | 46 (64) |
| INR, median (IQR) | 1.8 (0.6) | 1.9 (0.7) |
| Total Bilirubin, median (IQR) | 8.9 (19.3) | 5 (7.7) |
| Creatinine, median (IQR) | 2.1 (2.4) | 1.5 (2) |
| Albumin, median (IQR) | 3.2 (0.9) | 2.9 (1) |
| MELD, median (IQR) | 27 (11) | 24 (11) |
| At transplant | | |
| *n* | 19 | 38 |
| Age, median (IQR) | 56 (17) | 55 (13) |
| Male, *n* (%) | 13 (68) | 26 (68) |
| INR, median (IQR) | 1.6 (0.5) | 1.9 (0.8) |
| Total Bilirubin, median (IQR) | 8.3 (12.1) | 6 (5.7) |
| Creatinine, median (IQR) | 2.6 (2.9) | 1.3 (1.3) |
| Albumin, median (IQR) | 2.9 (0.8) | 2.8 (1) |
| MELD, median (IQR) | 24 (13) | 25 (9) |

IQR: Inter-quartile range; INR: International normalized ratio; MELD: Model for end stage liver disease.

### *Survival outcome and recidivism*

Both patients and controls had a median follow-up of more than three years after transplant (Table 3). One year after transplant 90% ± 7% of patients were alive compared with 92% ± 5% of controls. At five years, 73% ± 12% of patients was still alive compared with 77% ± 8% of controls (Figure 2). Survival rates after transplant did not differ significantly between patients and controls (log rank test, $P$-value = 0.837).

Among 19 patients who received their transplant based on OSOTC medically urgent exception criteria, four patients had resumed drinking by last follow-up visit for a 21% relapse rate after a median follow up of 3.4 years.

## DISCUSSION

For the first time, we report the rates of liver transplant and survival for patients with alcohol-induced cirrhosis who were deemed eligible for liver transplant and listed based on approval under the OSOTC medically urgent except criteria. These criteria allow patients with low to medium risk of recidivism, to receive a liver transplant after only one to three months of abstinence. These patients all committed to begin an alcohol treatment program before or during listing and to finish the program, even if it was after their transplant. We showed that transplant rate and short and long term survival after transplant is comparable between these patients and United States general population of patients with alcohol-induced cirrhosis who received their transplant after being eva-

**Table 3 Follow-up details of listed and transplanted patients and controls**

| | Patients | Controls | *P*-value |
|---|---|---|---|
| After listing | | | |
| No. listed | 36 | 72 | |
| No. transplanted (%) | 19 (53) | 41 (57) | 0.687[1] |
| Waiting time for transplant, d, median (IQR) | 19 (7-65) | 21 (5-54) | 0.648[2] |
| After transplant | | | |
| No. transplanted | 19 | 38 | |
| Follow-up after transplant, months, median (IQR) | 41 (29-58) | 37 (14-61) | |
| 1-yr survival, % ± SE | 90 ± 7 | 92 ± 5 | 0.837[3] |
| 5-yr survival, % ± SE | 73 ± 12 | 77 ± 8 | |

[1]$\chi^2$ test; [2]*P*-value for patient *vs* control time to transplant Cox proportional hazards model; [3]Log-Rank test. IQR: Inter-quartile range; SE: Standard error.

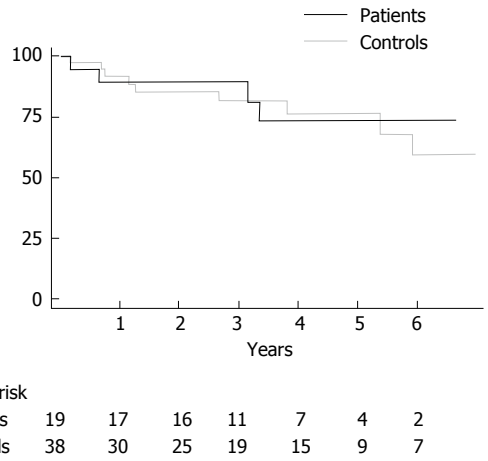


**Figure 2 Kaplan–meier estimates of survival after liver transplant in the 19 study patients and the 38 matched controls.**

luated for risk of recidivism based on conventionally used criteria. The risk of recidivism in our patients was comparable to previously published rates ranging from 15% to more than 20%[14-17].

Our findings challenge the notion of a defined abstinence period as the only criterion for liver transplant eligibility in patient with alcoholic liver cirrhosis[18]. However, the stringency of OSOTC process resulted in our selecting a very small number of patients with alcoholic cirrhosis for liver transplantation. Numerous studies have observed that the enforcement of sobriety period delays listing for transplantation in a significant number of patients with a low probability of alcohol relapse following liver transplant[17,19-23]. Indeed, the duration of alcohol abstinence before liver transplant is a poor indicator of relapse of alcoholism following transplantation[24].

Although our results are encouraging, the study has several limitations. The number of patients included in the study was small. Matched controls may not have been comparable to OSOTC patients in terms of family support, intention to remain abstinent from alcohol, or availability of counseling services at transplant center in the event of alcohol relapse. Future studies will benefit from a control group of patients with alcoholic liver disease undergoing liver transplantation that are matched to OSOTC patients on the basis of social and familial support.

In summary, liver transplantation may be an appropriate rescue option for selected patients with alcoholic liver disease after only one to three months of abstinence. Our results show that OSOTC transplant eligibility criteria provide a valid method to identify these patients who may benefit from liver transplantation with low to medium risk of recidivism.

## COMMENTS

### Background

A minimum of 12 mo of abstinence, or three months of abstinence and participation in an alcohol rehabilitation program, are the standard requirements before patients with alcoholic liver disease are eligible for transplantation in Ohio. Some patients are too ill to participate in a rehab program. The Ohio Solid Organ Transplantation Consortium (OSOTC) has a mechanism to evaluate risk of recidivism on a case-by-case basis.

### Research frontiers

Liver transplantation imparts great survival benefit to appropriately selected patients with advanced and de-compensated cirrhosis due to alcohol consumption, which is comparable to survival benefit of transplant in other types of chronic liver disease.

### Innovations and breakthroughs

The aim of this study was to determine the effect of using OSOTC transplant eligibility criteria on patients' survival compared with conventional criteria for assessment of risk of recidivism, in patient with cirrhosis due to alcoholic liver disease.

### Applications

Patients can be approved as an exception and listed for transplant after only one to three months of abstinence.

### Terminology

They were matched based on gender, age, and MELD score to controls with alcohol-induced cirrhosis from Organ Procurement and Transplant Network data.

### Peer-review

The manuscript "Ohio solid organ transplantation consortiumcriteria for liver transplantation in patients with alcoholic liver disease" by Hajifathalian *et al* is an interesting paper and the important contribution from OSOTC group supporting an update of the Criteria for Liver Transplantation based on alcohol abstinence duration potentially help to obtain the transplant for larger group of patients with a low probability of alcohol relapse.

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LETTERS TO THE EDITOR

# Is MELD score failing patients with liver disease and hepatorenal syndrome?

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## Abstract

There is a need to reassess the application of MELD and the impact of renal insufficiency with consideration for developing an algorithm with exception points that would lead to timely allocation of livers to patients with hepatorenal syndrome prior to occurrence of permanent renal damage without jeopardizing post-transplant survival.

**Key words:** MELD; Hepatorenal syndrome; Cirrhosis; Graft survival; Liver allocation



**Core tip:** The decompensation of patients with cirrhosis is associated with the development of hepatorenal syndrome (HRS) and renal insufficiency. There are several consequences of a high serum creatinine level in cirrhotic patients, including increased post - liver transplant mortality and increased risk of non-reversal of renal insufficiency/renal failure. We propose a change to the MELD scoring that would lead to timely liver transplantation in patients with HRS.

Sibulesky L, Leca N, Blosser C, Rahnemai-Azar AA, Bhattacharya R, Reyes J. Is MELD score failing patients with liver disease and hepatorenal syndrome? *World J Hepatol* 2016; 8(27): 1155-1156 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i27/1155.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i27.1155

## TO THE EDITOR

The decompensation of patients with cirrhosis is associated with the development of complications. This physiology can lead to renal hypoperfusion which contributes to the development of hepatorenal syndrome (HRS) and renal insufficiency[1,2]. It is rare to develop HRS with well-compensated liver disease.

There are several consequences of a high serum creatinine level in cirrhotic patients.

Serum creatinine is one of the most important independent predictors of waitlist and post-liver transplant (LT) mortality. While having the same MELD score, patients with higher serum creatinine level have a significantly higher mortality rate[3]. Analysis of the Scientific Registry of Transplant Recipients database linked with Centers for Medicare and Medicaid Services' end-stage renal disease (ESRD) data by Sharma *et al*[4] demonstrated that post-LT ESRD is associated with higher post-LT mortality (HR = 3.32; $P < 0.0001$).

Serum creatinine prior to liver transplantation is one of the most significant predictors of post-liver transplantation ESRD[5]. Wong *et al*[6] recently demonstrated that the only predictor of type 1 HRS non-reversal was the duration of pre-transplant dialysis with a 6% increased risk of non-reversal with each additional day of dialysis. Prolonged ischemic physiology may lead to structural renal damage and thus, prevent renal recovery. This has led many to consider combined liver-kidney transplantation (CLKT) for patients whose HRS has lasted longer than 6 wk because the outcomes for patients who receive CLKT seem to be better than those of patients who receive a liver transplant alone[7,8]. Since the introduction of MELD score, the number of patients treated with CLKT has increased markedly[9]. Almost 1000 kidneys a year are used in a combined transplantation, thus, diminishing the donor pool for patients on the kidney list.

It has also been shown that patients with renal insufficiency have longer hospital and intensive care unit stays and an increased need for dialysis, which likely increases the cost of transplantation. It likely adds to already increased healthcare costs through additional dialysis cases, and increased hospitalization rates secondary to morbidities associated with ESRD[10].

While MELD score is the gold standard for predicting wait list mortality, a notable weakness for liver allocation lies in predicting post transplantation survival, particularly with renal insufficiency[11,12]. In addition to MELD, various scoring systems, including Child Pugh score, the risk, injury, failure, loss, end-stage kidney disease criteria, sequential organ failure assessment (SOFA) score, and the Chronic Liver Failure-SOFA score have been designed to predict outcomes in post liver transplant patients[13]. Without a timely liver transplant for patients with acute kidney injury, the patient mortality is shifting from the waitlist to the post-transplant period[14]. It is time for a conversation within the transplant community to reassess the application of MELD and the impact of renal insufficiency with consideration for developing an algorithm with exception points that would lead to timely allocation of livers to patients with HRS prior to occurrence of permanent renal damage without jeopardizing post-transplant survival.

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