Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY



# The metabolomic window into hepatobiliary disease

Diren Beyoğlu, Jeffrey R. Idle*

*Hepatology Research Group, Department of Clinical Research, University of Bern, Bern, Switzerland*

## Summary

The emergent discipline of metabolomics has attracted considerable research effort in hepatology. Here we review the metabolomic data for non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), alcoholic liver disease (ALD), hepatitis B and C, cholecystitis, cholestasis, liver transplantation, and acute hepatotoxicity in animal models. A metabolomic window has permitted a view into the changing biochemistry occurring in the transitional phases between a healthy liver and hepatocellular carcinoma or cholangiocarcinoma. Whether provoked by obesity and diabetes, alcohol use or oncogenic viruses, the liver develops a core metabolomic phenotype (CMP) that involves dysregulation of bile acid and phospholipid homeostasis. The CMP commences at the transition between the healthy liver (Phase 0) and NAFLD/NASH, ALD or viral hepatitis (Phase 1). This CMP is maintained in the presence or absence of cirrhosis (Phase 2) and whether or not either HCC or CCA (Phase 3) develops. Inflammatory signalling in the liver triggers the appearance of the CMP. Many other metabolomic markers distinguish between Phases 0, 1, 2 and 3. A metabolic remodelling in HCC has been described but metabolomic data from all four Phases demonstrate that the Warburg shift from mitochondrial respiration to cytosolic glycolysis foreshadows HCC and may occur as early as Phase 1. The metabolic remodelling also involves an upregulation of fatty acid β-oxidation, also beginning in Phase 1. The storage of triglycerides in fatty liver provides high energy-yielding substrates for Phases 2 and 3 of liver pathology. The metabolomic window into hepatobiliary disease sheds new light on the systems pathology of the liver.


## Metabolomics and the liver in brief

Over the past decade or more, many authors have defined the terms metabolomics and metabonomics. It is unproductive and unnecessary to add further to these definitions here. All the reader needs to know from the point of view of hepatobiliary disease, is that metabolomics is a window that offers a view distinct from the lenses of genomics, transcriptomics, and proteomics. There can be no other organ where such a plethora of both lipids and water-soluble metabolites are metabolically interchanged. No other organ exceeds the rates of metabolism and energy production and consumption as found in the liver. Not only is the liver the source of myriad endogenous metabolites and precursors used by other organs, but also houses a vast array of detoxication enzymes that are crucial for rendering less toxic, more water-soluble and readily excretable the 1–3 million xenobiotics to which we are exposed in our lifetimes [1]. The hepatic metabolome is therefore a highly complex and dynamic flux of small metabolites (say, <1.5 kDa, to include the larger phospholipid species, such as cardiolipins). Metabolomics in its practice combines high-throughput analytical chemistry, typically, methodologies based upon mass spectrometry or nuclear magnetic resonance spectroscopy, with multivariate data analysis. These technologies permit comparison of "global" metabolite profiles in an "unbiased" fashion for two or more groups of samples. Of course, no metabolomic investigation has ever delivered a global metabolite profile for a sample set, as this would require employment of multiple analytical platforms and several sample preparation protocols that performed from millimolar down to subpicomolar concentrations. Moreover, different analytical platforms combined with specific sample preparation procedures each provide a different metabolomic window in the metabolic life of the liver. Accordingly, metabolomic findings reported are always biased by the laboratory analytical procedures employed, often highly so.

This notwithstanding, many metabolomic investigators in recent years have entered the field of hepatobiliary disease and a considerable volume of publications has appeared. This review is therefore timely and we will attempt to make sense of a large and heterogeneous set of published studies concerning the varied hepatobiliary elements of pathophysiology where metabolomics has had something to say. This metabolomic window on hepatobiliary disease has furnished an overabundance of potential disease biomarkers. More importantly, in our view, the metabolomic lens has begun to provide new insights into liver disease mechanisms, new understandings that may unmask potential therapeutic targets and, one day, new treatment modalities.

Keywords: Metabolomics; NASH; Cirrhosis; NAFLD; Hepatocellular carcinoma; Core metabolomic phenotype; Metabolic remodelling; Warburg effect.
*Received 7 April 2013; received in revised form 14 May 2013; accepted 21 May 2013*
* Corresponding author. Address: Hepatology Research Group, Department of Clinical Research, University of Bern, Floor F, Murtenstrasse 35, 3010 Bern, Switzerland. Tel.: +41 79 446 24 24; fax: +41 31 632 49 97.
*E-mail address:* jeff.idle@ikp.unibe.ch (J.R. Idle).

## The metabolomic window into non-alcoholic diseases of the liver

*Overview*

In this review and as depicted in Fig. 1, we will describe the extent to which metabolomics has informed on the progression from the healthy liver to hepatocellular carcinoma (HCC) through the various phases of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and liver cirrhosis. We will also examine what metabolomics has taught about the various influencing factors and putative risk factors for these diseases, such as obesity, diabetes, alcohol, hepatitis B and C virus (HBV, HCV) infection. In addition, we will also review what metabolomics has contributed to the understanding of the change in hepatic function after liver transplantation.

*Non-alcoholic fatty liver disease (NAFLD)*

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition that affects 15% to 45% persons in developed nations [2] and both children and adults from all ethnic groups [3]. A diagnosis of NAFLD implies an increased risk of such diseases as cardiovascular disease, diabetes, colonic adenomas, hypothyroidism, and polycystic ovary syndrome [3]. NAFLD is generally considered to be the hepatic manifestation of metabolic syndrome [4]. The reference standard for diagnosing hepatic steatosis remains liver biopsy [3]. Investigators have employed metabolomic protocols in an attempt to define biomarkers that might replace this invasive procedure for a disease of such high prevalence. Table 1 shows a summary of 11 studies with metabolomic components that inform regarding the formation of hepatic steatosis. Animal models and studies in living human subjects and human tissues have been employed. One common finding is that of increased lipid species in the liver and serum/plasma, including cholesterol esters [5,6], triacylglycerols [4–7], diacylglycerols [4], sphingomyelins [4], various bile salts [8–10], together with lactate [9,11,12] and glutamate [11,13]. In addition, cysteine-glutathione disulfide and both oxidized and reduced glutathione were all reported to be depressed in the liver and serum/plasma [8,9]. Finally, where diets that instigate fatty liver had been used, depressed concentrations of glucose were reported both in rat liver [14] and mouse serum [11], but in one study, elevated plasma glucose was reported [12]. Taken together with elevated mouse serum/plasma lactate [11,12], pyruvate and alanine [12], and human plasma lactate [9], these results would suggest that NAFLD engages in cytosolic glycolysis. NAFLD is frequently associated with insulin resistance and insulin has been reported in mice to activate pyruvate kinase M2 [15], the enzyme switch to glycolysis involved in the Warburg effect and thus the production of lactate and alanine from glucose via pyruvate. Furthermore, the reduction in glutathione derivatives in human liver [8] and plasma [9] in NAFLD is a clear sign of active oxidative stress in the liver.

The lipidomic component of the observations summarized in Table 1 is of interest. Firstly, it has been reported that phospho-

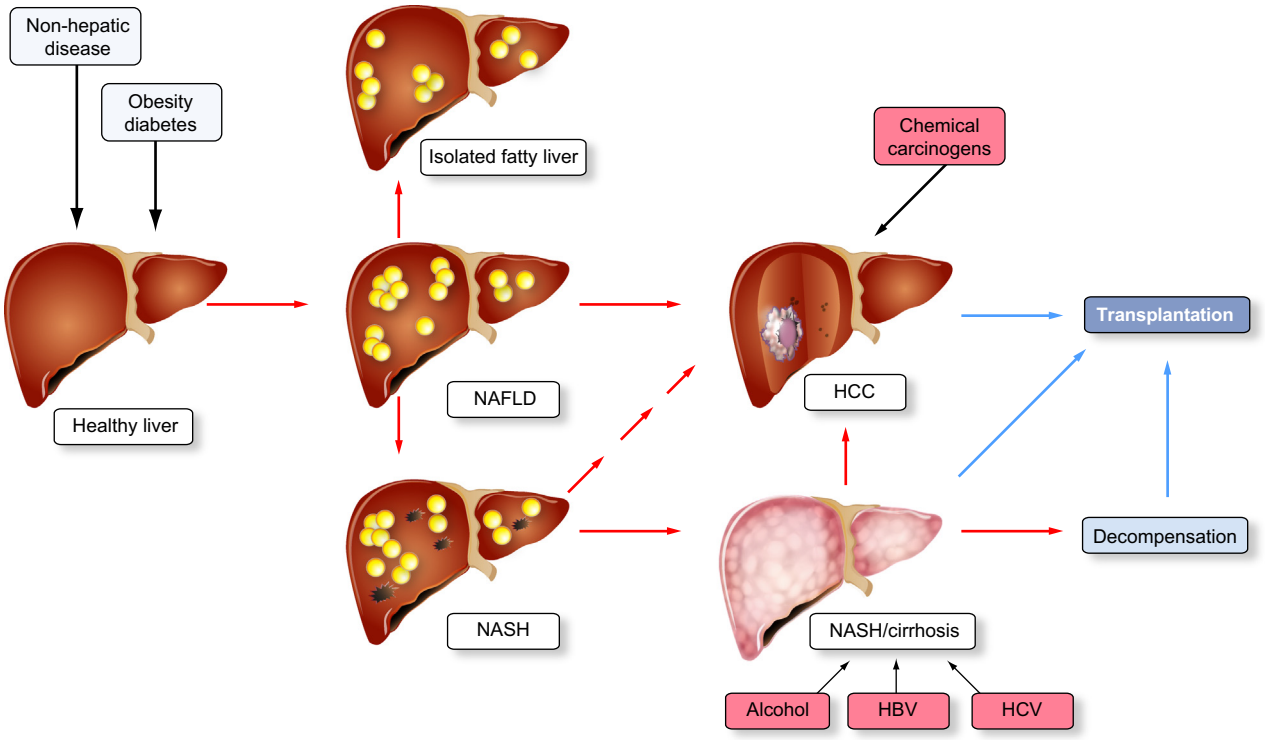


**Fig. 1. Major liver diseases and potential influencing factors.** This schematic shows the development of NAFLD from a healthy liver and various influencing factors. Steatosis is shown in yellow. NAFLD mostly becomes isolated fatty liver, but some cases progress to NASH, showing both steatosis and inflammatory necrosis (shown in red and black). NASH may progress to cirrhosis and then to HCC or to HCC directly. HCC, cirrhosis, and decompensated cirrhosis may all be treated by liver transplantation. Chemical carcinogens, such as aflatoxin $B_1$, together with alcohol and HBV and HCV infection, are all potential influencing factors. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.