Ketones and Terpenes in Exhaled Breath as Prospective Biomarkers for Chronic Liver Diseases

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1. Background and Objectives

Cirrhosis is the end-result of chronic hepatic injury, either associated with obesity and type 2 diabetes or exposure to damaging agents (e.g. alcohol abuse) (**Figure 1**). Early disease is often asymptomatic, with half of cases diagnosed at an advanced stage during an episode of decompensation, at which point damage is considered irreversible.

There is a pressing need for diagnostic tests allowing early detection of hepatic disease, both to screen susceptible populations but also to enable drug discovery.

Study design:

A cross-sectional, single-site study including healthy controls and subjects diagnosed with hepatocellular carcinoma (HCC), cirrhosis, or colorectal cancer with liver metastasis (CLRM).

Study demographics are shown at a high level in **Table 1.**

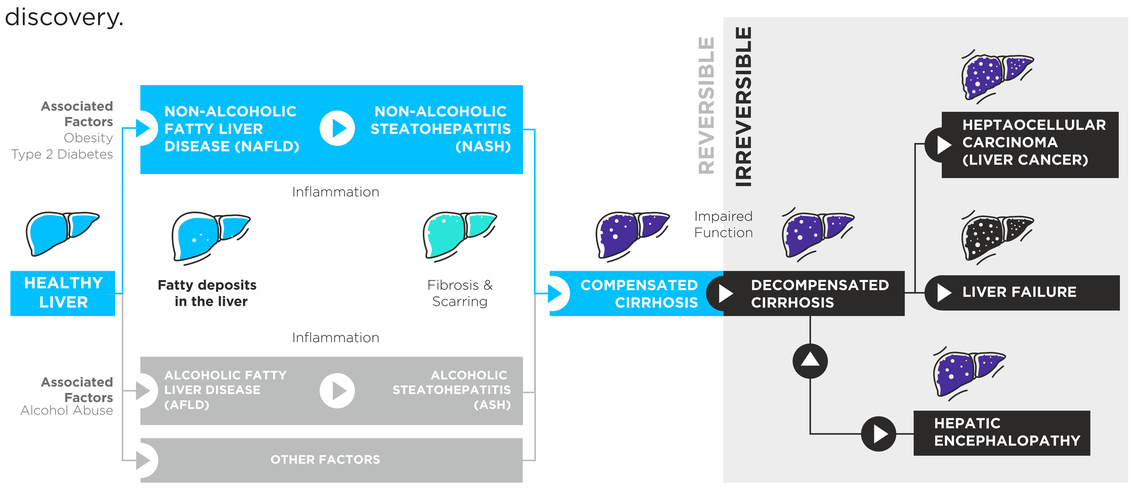

Figure 1: An overview of chronic liver diseases and their progression from healthy, through reversible disease states to decompensated.

Group	Sex		Age			
	Female	Male	Mean	Std. Dev.	Min	Max
Control	10	7	40.7	9.4	25	55
HCC	1	11	63.9	13.5	48	94
Cirrhosis	5	10	59.0	7.9	40	67
CLRM	2	6	58.4	12.3	42	76

Table 1: Summary demographic information for healthy controls and liver disease cases included in this study.

2. Methods

Breath Biopy samples were collected using the ReCIVA® Breath Sampler (**Figure 2**), developed by Owlstone Medical, and analysed with thermal desorption gas chromatography mass spectrometry (TD-GC-MS).

Relative quantification of VOCs was possible by comparison to eight deuterated internal standard compounds.

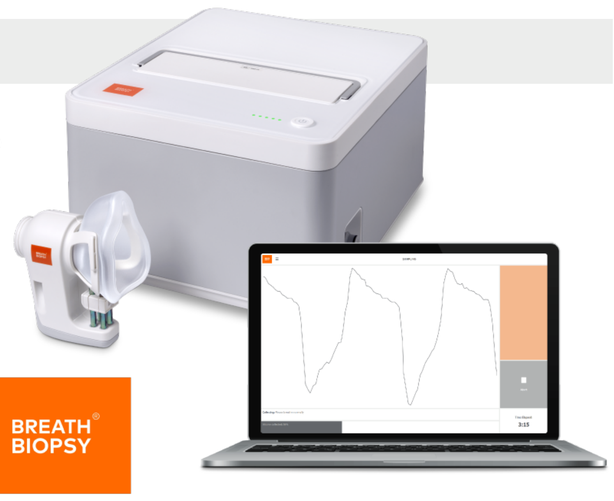

Figure 2: The Breath Biopsy® Collection Station, consisting of ReCIVA® Breath Sampler (left), CASPER® Portable Air Supply (top) and Breath Biopsy Collect Software (lower right).

3. Dataset Overview and Data Integrity

Assessment of data structure using Principal Component Analysis (PCA) showed a separation of a subset of subjects with cirrhosis and HCC from the core group of healthy controls (delimited with a dotted grey line) (**Figure 3**). Further evaluation suggested some contribution of model for end-stage liver disease (MELD) score to this separation.

The Mann-Whitney U-test was used to evaluate differences between groups. Since this is a small-scale pilot study, uncorrected p-value < 0.05 was used as a threshold for significance.

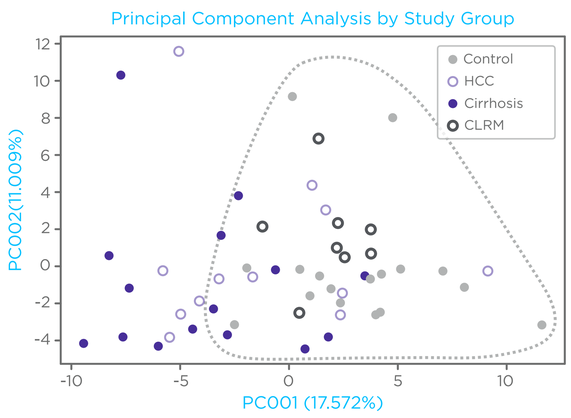

Figure 3: Principal Component Analysis (PCA) of collected breath sample data coloured by study group. Grey outline indicates the region defined by the range of health controls with a significant number of HCC and cirrhosis patients falling outside this zone.

4. Prospective Biomarkers Arising from Breath Analysis

Terpenes

Prior work in breath (Ferrandino et al[1], Fernandez del Rio et al.[2], among others) has shown associations between terpenes (limonene in particular) and hepatic damage.

Two features tentatively identified as terpenes by comparison to NIST library (MF91, MF78) replicated these observations, showing increased abundance in HCC or cirrhosis cases (**Figure 4**).

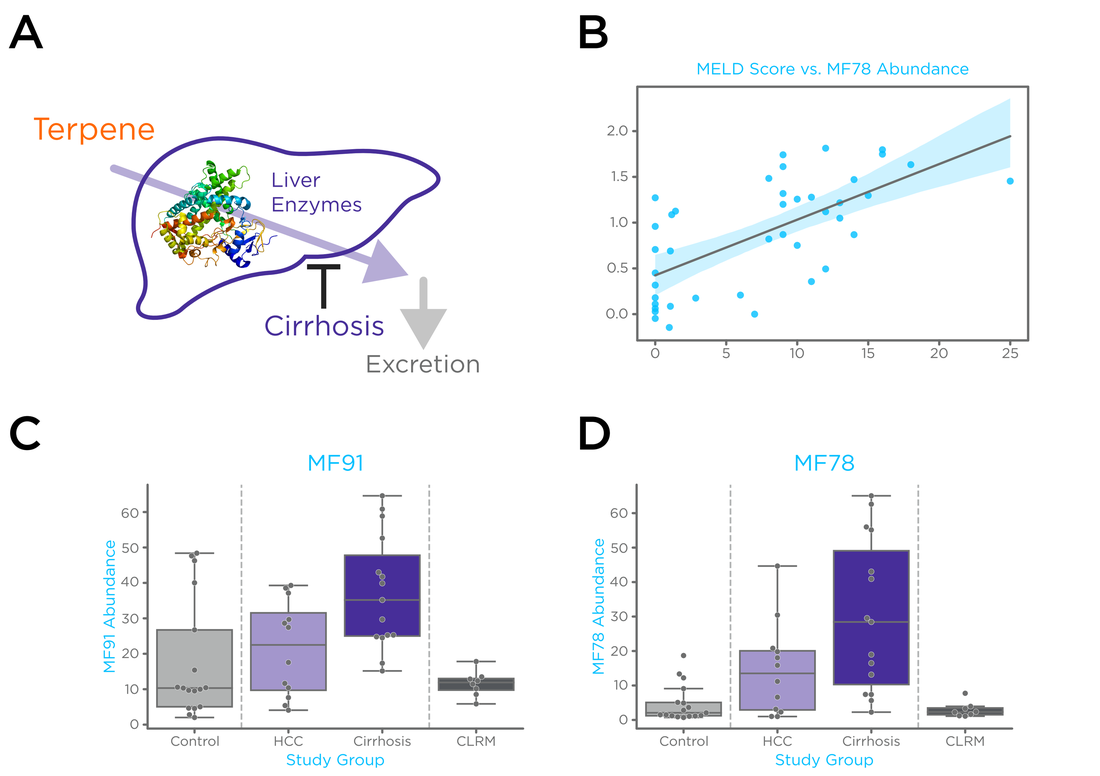

Figure 4: Terpene compounds measured on breath are typically metabolized by Cytochrome P450 enzymes in the liver. (A) Schematic representation of terpene metabolism in the liver. (B) Relationship between terpene MF78 and the MELD score for end-stage liver disease. Boxplots representing the relative abundances of terpenes MF91 (C) and MF78 (D) by study group.

Ketones

Links between ketones and liver diseases, have also been described in the literature[2]. Here, features tentatively identified as straight-chain ketones were associated with cirrhosis and HCC (**Figure 5**).

Biological source of these changes is uncertain; these changes could be associated with mitochondrial dysfunction in the liver, either due to impairment in beta-oxidation or compensatory changes in peroxisomal lipid oxidation.

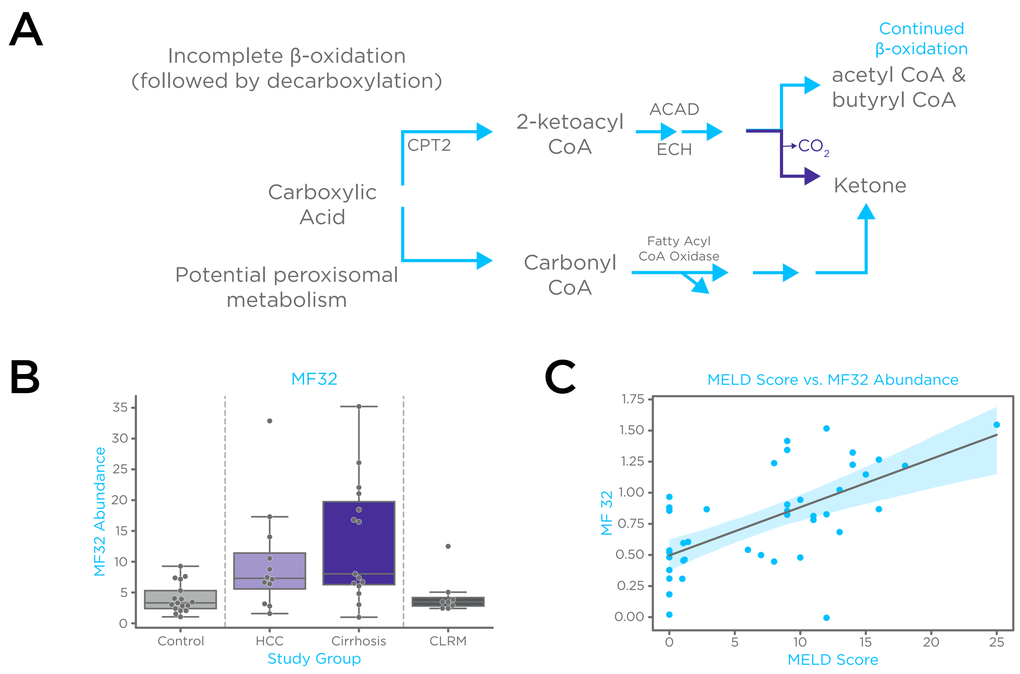

Figure 5: Ketone compound MF32 measured on breath are may be products of various stress and inflammation pathways. (A) Schematic representation of MF32 pathways. (B) Boxplots representing the relative abundances of MF32 by study group. (C) Relationship between MF32 and the MELD score for end-stage liver disease.

5. Conclusion

Here, we have used non-invasive sampling of breath volatiles, using Breath Biopsy, to identify candidate features associated with hepatic disease.

At a high-level, compound classes identified are consistent with prior findings in the literature, highlighting their importance and relevance as candidate biomarkers.

Sample size was limited in this study and further work to replicate and extend these findings is needed. In conclusion, this work provides further proof-of-principle evidence to support continued research on breath biomarkers in hepatic disease.

Further Resources

More about Breath Biopsy for Liver Diseases
owlstonemedical.com/liver-disease

Breath Biopsy: The Complete Guide (3rd Edition)
owlstonemedical.com/breath-biopsy-guide

Breath Biopsy Products & Services
owlstonemedical.com/products

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References

1. Ferrandino et al. Breath Biopsy Assessment of Liver Disease Using an Exogenous Volatile Organic Compound—Toward Improved Detection of Liver Impairment. *Clinical and Translational Gastroenterology*. 2020;11(9):e00239.
2. Fernandez Del Rio et al. Volatile Biomarkers in Breath Associated With Liver Cirrhosis - Comparisons of Pre- and Post-liver Transplant Breath Samples. *EBioMedicine*. 2015 Sep;2(9):1243-50.