BREATH® BIOPSY

TARGETED BREATH BIOPSY® PROFILING OF INDUCED BIOMARKERS UNVEILS A METABOLIC ADAPTATION IN CIRRHOSIS TOWARD ALCOHOL PRODUCTION

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Key Points

Subjects with cirrhosis showed increased presence of alcohol compared to healthy controls, which may be due to alterations of alcohol metabolism in the cirrhotic liver.

This alteration could explain increased blood alcohol concentration observed in abstinent subjects with cirrhosis and can be exploited for diagnostic purposes.

1. Background

Individuals with chronic liver disease exhibit elevated ethanol levels post carbohydrate intake, yet data on the liver's alcohol-handling capacity are sparse.

Exhaled breath analysis was employed in this study to investigate alcohol-ketone and ketone-alcohol conversion, aiming to discern metabolic changes and identify cirrhosis through a non-invasive breath test.

The research seeks to establish the utility of exhaled breath analysis in evaluating metabolic alterations and detecting cirrhosis, addressing a current gap in clinical understanding regarding liver function and metabolism in liver disease using different exogenous volatile organic compounds (EVOCs) as probes.

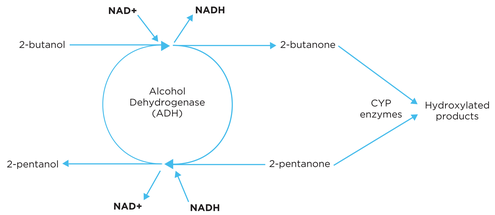


Figure 1. Reported or possible metabolic pathway for 2-butanol and 2-penantone. Alcohol dehydrogenase mediates the conversion of 2-butanol to 2-butanone reducing NAD+ to NADH. 2-pentanone could be converted to 2-pentanol by ADH. Other enzymes may be involved in this process. 2-butanone and 2-pentanone are further metabolized by the CYP enzymes.

2. Methods

The VOCs 2-butanol and 2-pentanone were chosen as substrates due to their safe profile for human consumption, documented hepatic metabolism, and presence in exhaled breath samples.

Hepatic metabolism of these compounds was validated by treating human hepatocyte suspensions with 2-butanol and 2-pentanone, measuring their conversion to 2-butanone and 2-pentanol using headspace analysis coupled with thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS).

Clinical metabolism was assessed in 14 cirrhosis patients and 15 controls by analyzing breath profiles before and at various time points (10 - 120 minutes) following simultaneous ingestion of 100 mg of 2-butanol and 2-pentanone, and other EVOCs, utilizing Breath Biopsy® OMNI.

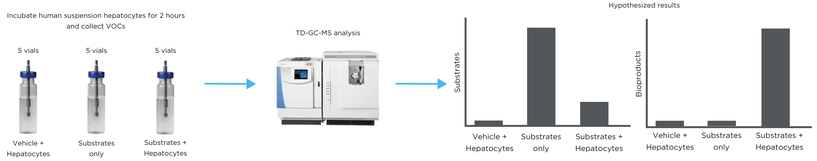
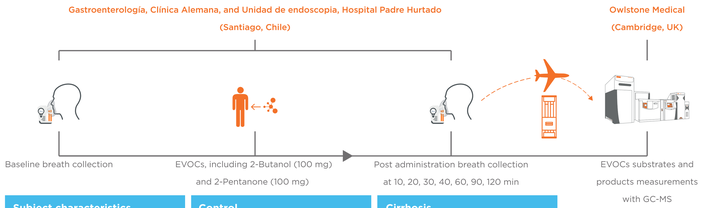


Figure 2. Study set-up to assess hepatic metabolism of investigated compounds. Deuterated 2-butanol and 2-pentanone, together with other EVOCs were incubated with primary hepatocytes (Hep) suspension in HiSorb vials for 2 hours at 37°C, then compounds were quantified using TD-GC-MS. Control conditions were Hep with vehicle, and substrates without Hep.



Subject characteristics		Control	Cirrhosis
Number		15	14
Age, median [Q1, Q3]		57.0 [46.0, 61.0]	57.0 [51.2, 61.5]
Gender, n (%)	M F	3 (20.0) 12 (80.0)	3 (21.4) 11 (78.6)
Body Mass Index (BMI), median [Q1, Q3]		138.9 [114.3, 169.5]	130.0 [122.3, 147.4]
MELD, median [Q1, Q3]		-	6.5 [6.0, 8.8]

Subject protocol:

- Fasted for ≥ 10 h
- Avoid confounders such as perfumes and lipsticks

Figure 3. Study design for evaluation of clinical hepatic metabolism. Controls and subjects with cirrhosis were asked to fast overnight. Breath was collected before and at different time points after ingestion of investigated compounds. Absolute quantification of analytes was obtained by TD-GC-MS by comparison with a calibration curve.

3. Results

The presence of bioproducts was confirmed in the headspace of primary hepatocytes suspension treated with the substrates, while control conditions lacking primary hepatocytes or substrates showed their absence.

Following an overnight fast, all participants exhibited detectable breath levels of the investigated compounds, with no significant differences observed between controls and those with cirrhosis ($p > 0.05$).

Upon ingestion of the substrates, a substantial increase (> 100-fold) in the investigated compounds was observed within 10 minutes in all subjects. Subjects with cirrhosis showed significantly higher levels of 2-butanol and 2-pentanol ($p < 0.05$) compared to controls between 20 and 90 minutes post administration. Additionally, an exploratory classification model utilizing breath-induced biomarkers at 20 minutes yielded an area under the ROC curve of 0.86, indicating promising diagnostic potential.

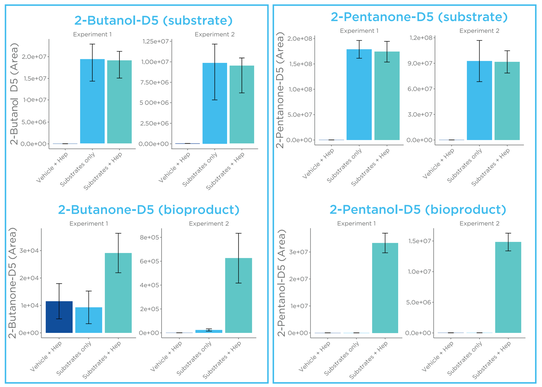


Figure 4. Expected bioproducts were present when substrates were incubated with primary hepatocytes. 2-butanol was converted to 2-butanone, and 2-pentanol was converted to 2-pentanone when the substrates were incubated in the presence of hepatocytes suspension. Control conditions omitting primary hepatocytes or substrates showed lack of significantly reduced conversion. D = deuterated.

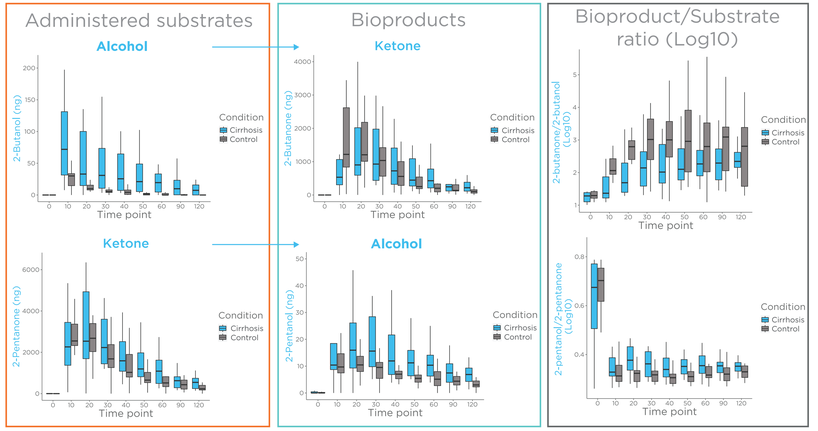


Figure 5. Breath profile of investigated compounds in subjects with cirrhosis, or cirrhosis-free controls. After an overnight fasting no significant differences in the investigated compounds were measured between controls and subjects with cirrhosis. Biomarkers induction via oral administration of EVOCs induced a spike in the breath in all the subjects. Cirrhosis subjects showed higher levels of conversion of ketone to alcohol, and lower conversion of alcohol to ketone compared to healthy controls. Ratio of Log10 (bioproduct)/Log10 (substrate) was calculated for each time point.

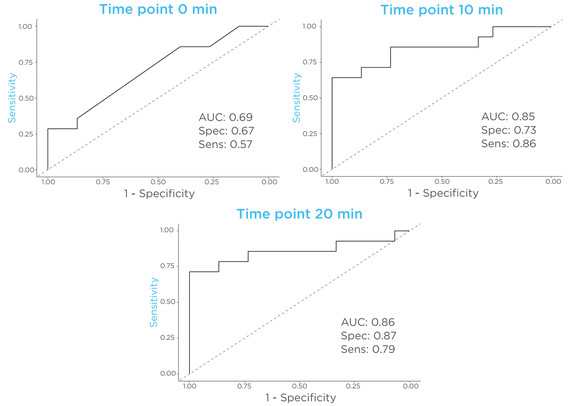


Figure 6. Classification performance of Bioproducts/Substrates ratios. An exploratory logistic regression model was generated to estimate potential classification performance for subjects with cirrhosis. Baseline showed poor classification performance, while biomarker induction showed improved performance shortly after administration.

4. Conclusions and Next Steps

2-butanol and 2-pentanone are metabolized to corresponding ketone and alcohol in primary hepatocytes, supporting previous findings on hepatic metabolism.

Subjects with cirrhosis exhibit higher breath levels of secondary alcohols, regardless of alcohol or ketone administration suggesting a metabolic adaptation.

Breath profile alterations of investigated EVOCs allow discrimination of subjects with cirrhosis from controls after biomarker induction.