

History of Alcohol Consumption Uniquely Increases Hepatic Hyaluronan Content in Patients with Liver Steatosis

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INTRODUCTION

Hyaluronan (HA), an extracellular matrix glycosaminoglycan, is increased in plasma from donors with advanced liver disease and is used as a biomarker to assess liver disease severity. Extrahepatic HA content and its fragmentation are associated with inflammation and/or fibrosis.

In chronic, persistent hepatitis, as compared to normal tissue, HA was increased in fibrous areas around the portal tracts and the central vein, but it was rarely detected in the sinusoidal walls. In chronic aggressive hepatitis, HA was present in periportal sinusoidal walls in addition to fibrous tissues of the portal tract (Ichida, 1996).

In cirrhotic tissues, as compared to normal, HA was significantly increased in fibrous areas of the portal tract but to a lesser extent in the sinusoidal walls around periportal areas (Ichida, 1996).

In alcoholic cirrhosis, HA was also observed in the sinusoidal wall and fibrous areas around the portal tract and central vein and its abundance in these locations diminished upon alcohol abstinence (Urashima, 1999).

Since hepatic levels and patterns of distribution of HA have not been examined in early liver disease (steatosis), we utilized liver tissue microarrays (TMAs) and histochemical staining with hyaluronan binding protein (HABP) to determine if hepatic HA increases in donors with steatosis and if HA content is affected by history of alcohol consumption.

MATERIALS & METHODS

Tissue donors for TMAs focused on steatosis without a history of alcohol consumption or steatosis with a history of alcohol consumption had similar age and sex distribution (Table 1). Macrovesicular steatosis was estimated from hematoxylin and eosin-stained (H&E) tissues by a pathologist. Body mass index (BMI) data were found in hospital records while information on alcohol consumption was provided by next of kin.

HA was visualized by HABP staining (McCracken JM, 2017).

Images of the arrays were obtained with a Nikon HCA system and a Hamamatsu Orca Flash 4 camera.

NIH Image J (v. 1.51) software was used to quantify HA-positive area in images from each core; upper and lower thresholds were kept the same for each core on both arrays. Hyaluronidase pre-treatment of liver tissues confirmed specificity of the HA localization reagent. Student's t-test was used to determine significance between two groups. One-way ANOVA was used to determine significance between 3 groups and was followed by a post-hoc analysis (Tukey's adjustment) for multiple comparisons. Significance was set as $P < 0.05$. All statistics were performed using GraphPad Prism v. 7.0 (San Diego, CA).

Table 1. Donor Pool

	n	Male, female	Age (years)	Diabetes
Normal controls	10	6, 4	Median 35, range 21 - 63	no
Steatosis without a history of alcohol consumption	20	13, 7	Avg 49.8 ± 13.9	8
Steatosis with a history of alcohol consumption	19	14, 5	Avg 51.4 ± 8.9	no

CONCLUSIONS

In livers from donors with macrovesicular steatosis, alcohol consumption was associated with elevated hepatic hyaluronan content by 3-fold. These data suggest etiology-specific regulation of hyaluronan accumulation in liver disease.

REFERENCES

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 McCracken JM, et al. 2017. *Hepatology*, 66(1):207A.
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RESULTS

Figure 1. BMI and Percentage of Hepatic Steatosis in Donors

Neither BMI nor hepatic steatosis were different between donors with or without a history of alcohol consumption. There was no difference in BMI between donors without or with a history of alcohol consumption [41.4 ± 3.6 (SEM) vs 33.6 ± 2.4] (left panel) or in macrovesicular steatosis (37.4 ± 5.2 vs 48.7 ± 7.0) ($P > 0.05$) (right panel). BMI and steatosis were significantly greater in donors with steatosis as compared with normal donors (ANOVA).

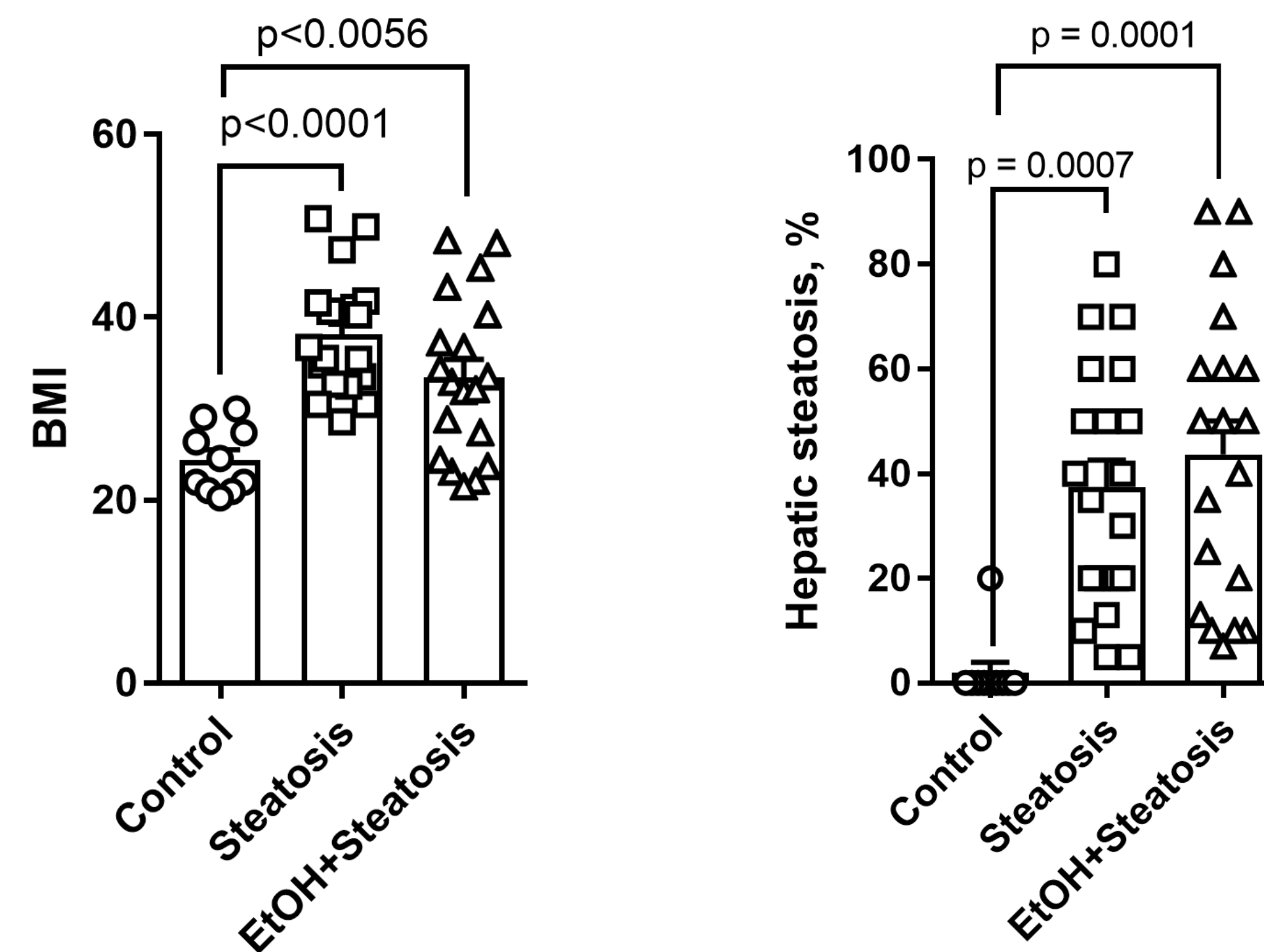


Figure 2. Hyaluronan Localization in Livers from Donors with Steatosis

In normal livers from donors without a history of alcohol consumption, hyaluronan was detected in periportal regions (top row). Hyaluronan was higher in livers from donors with steatosis and a history of alcohol consumption where a diffuse distribution of HA was also seen (e.g. H0575). All donors presented at the bottom row had a history of heavy alcohol consumption.

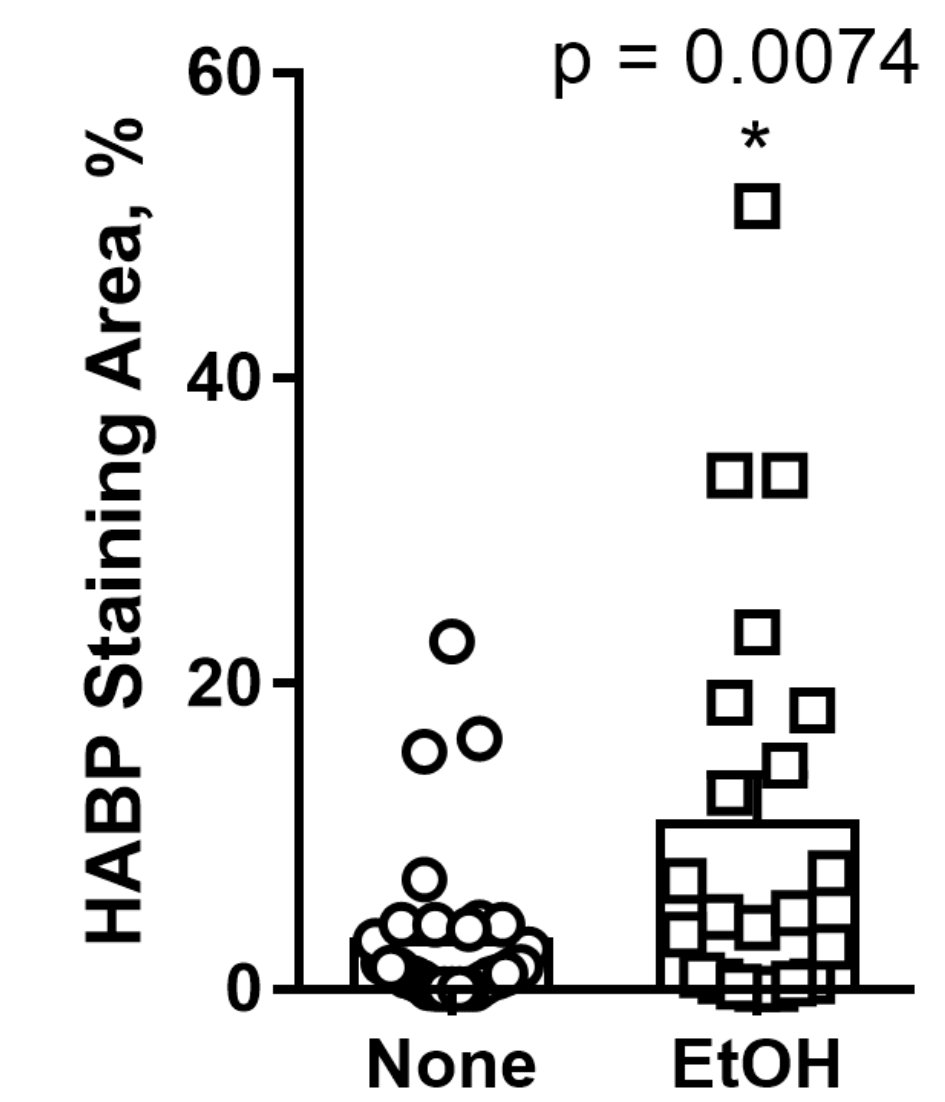
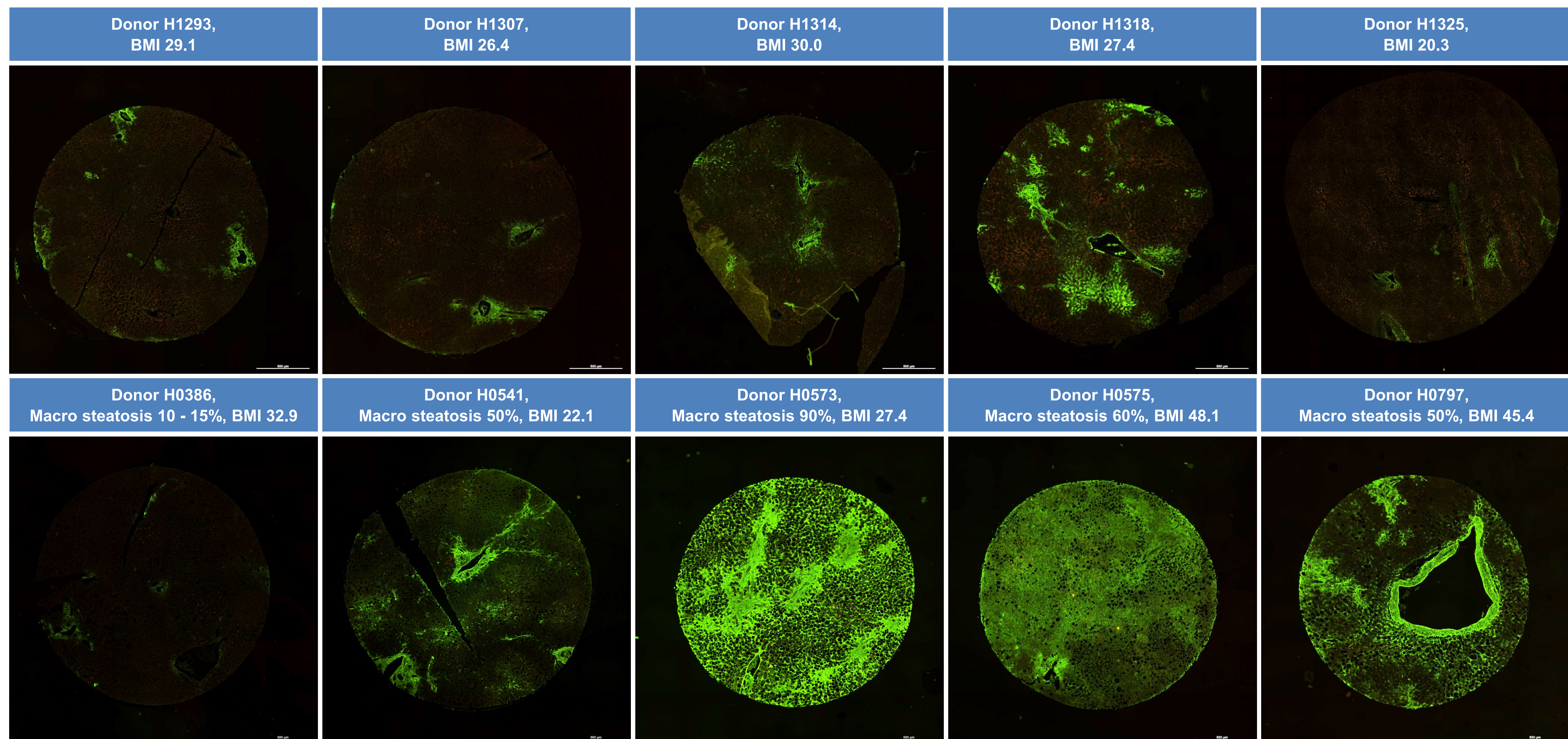


Figure 3. Hepatic Hyaluronan Content in Livers from Alcohol-Consuming or Non-Alcohol Consuming Donors

Hepatic hyaluronan content was greater in livers from donors who consumed alcohol compared to those who did not consume alcohol (11.1 ± 2.9 vs 3.4 ± 1.0 , t-test).

Figure 4. Hepatic Hyaluronan Accumulation in Healthy Donors, Donors with Steatosis and Donors with Steatosis and History of Alcohol Consumption

Hyaluronan preferentially accumulated in steatotic livers from donors who consumed alcohol relative to donors with steatosis who did not consume alcohol. Hyaluronan was about 3-fold greater in livers from donors with steatosis and a history of alcohol consumption as compared to donors without a history of alcohol consumption (ANOVA). There was no significant difference in HABP staining area between controls (non-steatotic, non-drinkers) compared to donors with steatosis who did not drink ($P > 0.05$).

