Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

JOURNAL OF HEPATOLOGY

Increased expression of key SARS-CoV-2 entry points in multiple tissues in individuals with NAFLD

To the Editor:

Recently, Fondevila and colleagues[1] reported that individuals with non-alcoholic steatohepatitis (NASH), one of the more severe manifestations of non-alcoholic fatty liver disease (NAFLD), have increased hepatic expression of key entry points of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Increased hepatic expression of the SARS-CoV-2 entry points angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) in individuals with NAFLD possibly provides a mechanistic explanation for their increased susceptibility to hepatic complications. These findings are of relevance during the ongoing coronavirus disease 2019 (COVID-19) global health crisis, which has particularly affected those with underlying health conditions including NAFLD.[2–4] Given the multifactorial and multi-organ nature of NAFLD, herein, we assess the multi-organ expression of SARS-CoV-2 entry points in tightly matched obese individuals with and without NAFLD.

To assess multi-organ expression of SARS-CoV-2-entry points in NAFLD, we included 56 women from our bariatric surgery cohort[5] of whom RNA transcriptomic data was available from the liver, jejunum, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Prior to surgery, every individual underwent a complete metabolic work-up (Table S1) including a 2-hour mixed meal tolerance test (MMT) to assess insulin resistance. In total, 23 individuals fulfilled the criteria for NAFLD (biopsy-proven) whereas 33 individuals did not. NAFLD ranged from grade 1 to grade 2 steatosis. Of note, none of the included individuals had hepatocyte ballooning, a prerequisite for NASH diagnosis according to the Steatosis Activity and Fibrosis (SAF) criteria. From the extensive list of baseline characteristics, only alanine aminotransferase was significantly higher in individuals with NAFLD. Moreover, insulin and glucose excursions during the MMT did not differ between groups (Fig. 1A and 1B). We hence have a very homogenous study population that differ only in presence or absence of NAFLD. *ACE2* expression was significantly higher in the SAT, VAT and liver of individuals with NAFLD (Fig. 1C–E) but did not reach significance in jejunal tissue (Fig. 1F). Next, we addressed expression of the proteases, *TMPRSS2*, *TMPRSS4* and *Furin* that assist ACE2-dependent cell entry of Sars-CoV-2. Although *TMPRSS2* appeared higher in the VAT and liver of individuals with NAFLD, expression levels did not reach significance (Fig. 1H and 1I). In all tissues, *TMPRSS2* and *ACE2* correlated strong with each other. *TMPRSS4* and *Furin* did not differ in the tissues analyzed (data not shown).

Since metabolic dysregulation has been shown to be an independent predictor of death and morbidity in previous infectious epidemics,[6] we investigated the correlation between SARS-CoV-2-entry points and components of insulin resistance. Interestingly, liver *ACE2* expression significantly correlated with insulin area under the curve (AUC) but not with glucose AUC determined during the MMT.

In conclusion, we report that *ACE2* is upregulated in the SAT, VAT and liver tissue of individuals with NAFLD. A presumably greater availability of ACE2 and the strong correlation with TMPRSS2 in these organs likely fosters viral penetration into cells. These results strengthen the obesity-independent link between NAFLD and severe COVID-19. Moreover, the strong correlation between insulin AUC and *ACE2* in the liver might explain adverse clinical outcomes and laboratory results in SARS-CoV-2-infected individuals with T2D on insulin compared to those on other glucose-lowering agents.[7,8] We thus presumably identified an additional mechanism that contributes to increased susceptibility to severe COVID-19 in individuals with NAFLD and individuals with insulin resistance or T2D. Last but not least, we underscore once more that “simple steatosis” is not as benign as sometimes assumed. Our results highlight the need to develop accurate, non-invasive diagnostic methods for the early detection of NAFLD and for subsequent COVID-19 risk stratification.

Financial support

The BARIA study is funded by the Novo Nordisk Foundation (NNF15OC0016798).

Conflict of interest

The authors declare no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

A.K.G, M.N and H.H. supervised this work, - A.S.M & H.H. conducted conceptual design, data curation, data analysis, visualization and main manuscript preparation. S.B & A.S.M collected medical data and biopsies.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.12.007.

References

[1] Fondevila MF, Mercado-Gómez M, Rodríguez A, Gonzalez-Rellan MJ, Iruzubieta P, Valentí V, et al. Obese patients with NASH have increased hepatic expression of SARS-CoV-2 critical entry points. J Hepatol 2021;74:469–471. https://doi.org/10.1016/j.jhep.2020.09.027.

[2] Ji D, Qin E, Xu J, Zhang D, Cheng G, Wang Y, et al. Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study. J Hepatol 2020;73:451–453. https://doi.org/10.1016/j.jhep.2020.03.044.

[3] Zhou YJ, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, et al. Metabolic-associated fatty liver disease is associated with severity of COVID-19. Liver Int 2020;40:2160–2163. https://doi.org/10.1111/liv.14575.

[4] Zhou YJ, Zheng KI, Wang XB, Yan HD, Sun QF, Pan KH, et al. Younger patients with MAFLD are at increased risk of severe COVID-19 illness: a multicenter preliminary analysis. J Hepatol 2020;73:719–721. https://doi.org/10.1016/j.jhep.2020.04.027.

Received 23 November 2020; accepted 6 December 2020; available online 16 December 2020
https://doi.org/10.1016/j.jhep.2020.12.007

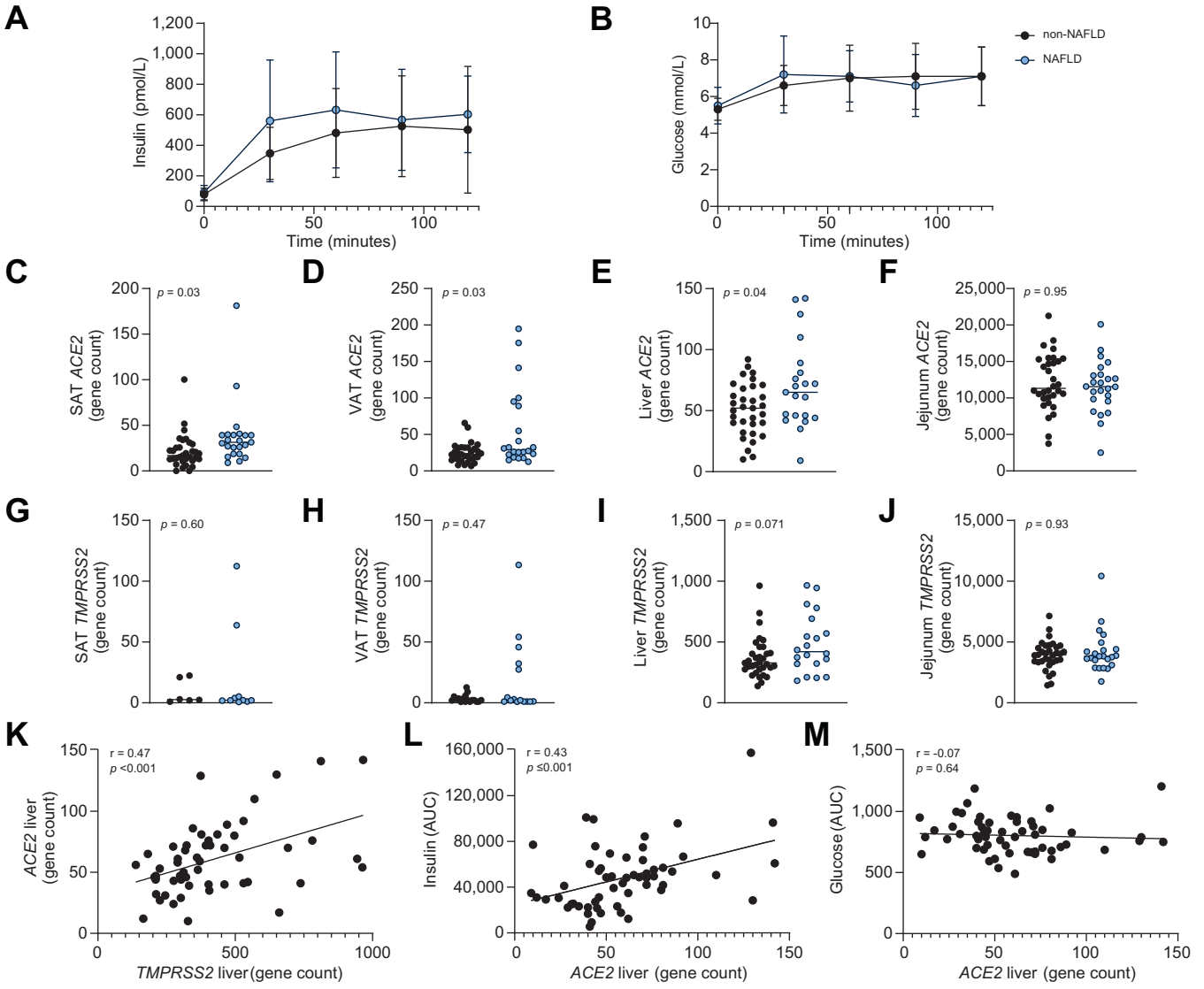

Fig. 1. SARS-CoV-2 entry point expression in patients with and without NAFLD. (A) Insulin and (B) glucose excursions during the MMT. (C–F) *ACE2* expression in individuals with and without NAFLD in different tissues. (G–J) *TMPRSS2* expression in individuals with and without NAFLD in different tissues. (K) Pearson correlation between hepatic *ACE2* and *TMPRSS2* expression. (L) Pearson correlation between hepatic *ACE2* expression and insulin AUC determined during the MMT. Pearson correlation between hepatic *ACE2* expression and glucose AUC determined during the MMT. For comparison between gene expression levels Mann-Whitney *U* test was used. ACE2, angiotensin converting enzyme 2; AUC, area under the curve; MMT, mixed meal tolerance test; NAFLD, non-alcoholic fatty liver disease; SAT, subcutaneous adipose tissue; TMPRSS2, transmembrane protease serine 2; VAT, visceral adipose tissue.

[5] Van Olden C, Van de Laar A, Meijnikman A, Aydin O, Van Olst N, Hoozemans JB, et al. A systems biology approach to understand gut microbiota and host metabolism in morbid obesity: design of the BARIA Longitudinal Cohort Study. J Intern Med 2020:1–15. https://doi.org/10.1111/joim.13157.

[6] Apicella M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: understanding the reasons for worse outcomes. Lancet Diabetes Endocrinol 2020;8:782–792. https://doi.org/10.1016/S2213-8587(20)30238-2.

[7] Chen Y, Yang D, Cheng B, Chen J, Peng A, Yang C, et al. Clinical characteristics and outcomes of patients with diabetes and COVID-19 in association with glucose-lowering medication. Diabetes Care 2020;43:1399–1407. https://doi.org/10.2337/dc20-0660.

[8] Yu Bo, Li Chenze, Sun Yang, Wang Dao-Wen. Insulin treatment is associatedwith increased mortality inpatients with COVID-19 and Type 2 Diabetes. Cell Metabol 2020;33:1–13. https://doi.org/10.1016/j.cmet.2020.11.014.

Abraham S. Meijnikman[1,*]
Sjoerd Bruin[2]
Albert K. Groen[1]
Max Nieuwdorp[1]
Hilde Herrema[1,*]

[1]*Departments of Internal and Experimental Vascular Medicine, Amsterdam University Medical Centers, Location AMC, Amsterdam, The Netherlands*

[2]*Department of Surgery, Spaarne Hospital, Hoofddorp, The Netherlands*

[*]Corresponding authors. Address: Amsterdam University Medical Centers, Location AMC, Experimental Vascular Medicine, lab G1-115 Meibergdreef 9, 1105AZ, Amsterdam, the Netherlands. Tel.: +31(0)20 5663224.

E-mail addresses: a.s.meijnikman@amsterdamumc.nl (A.S. Meijnikman), h.j.herrema@amsterdamumc.nl (H. Herrema)