Obese patients with NASH have increased hepatic expression of SARS-CoV-2 critical entry points

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Supplementary Material and Methods

Human liver samples

All reported investigations were carried out in accordance with the principles of the Declaration of Helsinki, as revised in 2013, and approved by the Hospital's Ethical Committee of each hospital (protocol 2017.104). Written informed consent was obtained from all the participants.

Liver samples were obtained from patients with obesity undergoing bariatric surgery (n=94) at the Clínica Universidad de Navarra and at the Marqués de Valdecilla University Hospital (MVUH, Santander, Spain). Inclusion criteria were based on alcohol intake lesser than 20 g/day for woman and lesser than 30 g/day for men, no evidence of hepatitis B and/or C virus infection as well as human immunodeficiency virus (HIV) and, no evidence of concomitant liver disease such as autoimmune liver diseases, haemochromatosis, Wilson's disease or α-1 antitrypsin deficiency. Comorbidities, anthropometric measures, laboratory data (alanine aminotransferase -ALT-, aspartate aminotransferase -AST-, γ-glutamyltransferase -GGT-, triglycerides, cholesterol, high-density lipoprotein cholesterol -HDL- and low-density lipoprotein cholesterol -LDL) and histology data were collected. The clinical characteristics of these patients are indicated in Supplemental table 1. Hepatic histopathological analysis was performed according to the scoring system of Kleiner et al. Simple steatosis was defined as the presence of at least 5% of steatotic hepatocytes with or without mild lobular or portal inflammation but in the absence of features of hepatocellular injury (ballooning,

apoptosis or necrosis) and fibrosis. On the other hand, minimal criteria for the histological diagnosis of definite steatohepatitis included the combined presence of grade 1 steatosis, hepatocellular injury and lobular inflammation with or without fibrosis.

RNA extraction and real-time PCR

RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA (100 ng) was used for each RT reaction, and cDNA synthesis was performed using the M-MLV Reverse Transcriptase (ThermoFisher) and random primers. Negative control reactions, containing all reagents except the sample were used to ensure specificity of the PCR amplification. Gene expression was determined by real-time quantitative PCR using SYBR Green PPLUS-LR-SY reagent (PrimerDesign) and a QuantStudio 5 Real-Time PCR Instrument (Applied Biosystems). Cycling conditions included an initial denaturation at 95ºC for 3 min followed by 40 cycles at 95°C for 5 seconds and 60°C for 32 seconds, with a holding stage of 95°C for 15 seconds, 60ºC for 1 min and 95ºC for 15 seconds. Amplification was done using oligonucleotide specific primers: Human *ACE2* forward primer 5´-GTGCACAAAGGTGACAATGG-3´ and reverse primer 5´-GGCTGCAGAAAGTGACATGA-3´; Human *HPRT* forward primer 5´-ACCCCACGAAGTGTTGGATA-3´ and reverse primer 5´-AAGCAGATGGCCACAGAACT-3´; Human *TMPRSS2* forward primer 5´-CTCTCCCTAACCCCTTGTCC-3´ and reverse primer 5´-AGAGGTGACAGCTCCATGCT-3´. All reactions were performed in duplicate. Expression levels were normalized to *HPRT* for each sample and expressed in relation (%) to the control group.

Statistical analysis

Data are expressed as mean ± standard error mean (SEM). To test if the populations follow a Gaussian distribution, a normality test was performed (Kolmogorov–Smirnov test for n between 5 and 7; Shapiro–Wilk test for $n \geq 7$). For normal distributions, a parametric test was used: for two population comparisons, an unpaired two-tailed Student´s *t*-test was used; for multiple comparisons, a one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* multiple comparison test was performed. For non-Gaussian distributions, the following were used: Mann–Whitney *U* tests were used for two comparison tests and Kruskal–Wallis followed by Dunn *post hoc* test for multiple comparisons. $P < 0.05$ was considered significant for all the analysis. Analyses were performed with the Prism Software Version 6.0 (GraphPad). Pearson's (parametric) or Spearman's (nonparametric) correlation coefficient (r) was used to study the correlation between gene expression and different parameters.

Supplementary tables.

Table S1. **Anthropometric, biochemical and clinical characteristics of obese patients.** BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; HOMA, homeostasis model assessment.

	Without NALFD	**Steatosis**	**NASH**
n	17	57	20
Age (years, mean ± SD)	43.1 ± 8.7	46 ± 10.9	49.2 ± 10.4
Gender (F / M)	12 / 5	29 / 28	11 / 9
Weight (kg ± SD)	116.3 ± 27.8	123.1 ± 22.9	137 ± 23.7
BMI	40.2 ± 7.7	43.8 ± 6.3	48.3 ± 6.8
LDL (mg/dl ± SD)	107.8 ± 33.8	112.5 ± 33.5	105.9 ± 32.2
HDL (mg/dl ± SD)	48.3 ± 12.6	45.8 ± 15.4	42.5 ± 11.6
Triglycerides (mg/dl ± SD)	102.6 ± 67.5	140.8 ± 65.9	161.4 ± 74
Cholesterol (mg/dl ± SD)	176.8 ± 41.2	188.9 ± 38.7	185.5 ± 36.1
AST (U/L ± SD)	17.6 ± 4.9	21.1 ± 6.2	36.3 ± 20.9
ALT (U/L ± SD)	21 ± 8.5	27.6 ± 13.3	44.6 ± 25.3
Glucose (mg/dl ± SD)	109.9 ± 55.4	110.8 ± 42.3	121.3 ± 38.5
Insulin (mU/ml ± SD)	12 ± 8.3	26.7 ± 41.5	17.8 ± 12.6
HOMA	3.7 ± 4.3	5.7 ± 3.7	5.5 ± 5
Type 2 diabetes	29.4%	47.4%	55%
NAS score ≥ 5	0%	15.8%	45%
Metabolic syndrome	35.3%	54.5%	78.9%