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Reply:

We thank Ballestri et al. for their comments about our recent and past publications.[1,2] We would like to remind them and our reading audience that the nonalcoholic fatty liver disease activity score (NAS) of the Nonalcoholic Steatohepatitis (NASH) Clinical Research Network was developed and validated for numerically describing the spectrum of nonalcoholic fatty liver disease. It was never intended to replace a pathologist's diagnosis of steatohepatitis, but this conclusion is implicit in the analysis undertaken by Ballestri et al. Because other authors have also misapplied the NAS to diagnose NASH, we undertook the study described in our 2011 publication[1] to determine the implications of doing this in a very large patient sample. Interestingly, we found that a high NAS correlated more with elevated alanine aminotransferase levels, whereas the diagnosis of NASH correlated more with features of metabolic syndrome.

A scoring system is meant for just that: scoring relevant lesions of an injury pattern so that the severity of the disease can be monitored semiquantitatively over time and in cohorts of patients, especially those in treatment trials. None of the scoring systems for chronic hepatitis, liver transplant rejection, and biliary diseases were developed to diagnose these diseases, and neither was the NAS. When we described the NAS,[2] we did note that there was a statistical correlation between an NAS value ≥ 5 and an independent diagnosis of steatohepatitis by a pathologist. Unfortunately, this observation has been misinterpreted by some as a proposal to replace the diagnosis with the score, even though we clearly stated otherwise in our study. Publications from the NASH Clinical Research Network have consistently separated the diagnosis from the score, and we have emphasized this separation in our presentations. We hope that other investigators will find it appropriate to make this distinction as well.

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Cyclooxygenase Inhibition Up-Regulates Liver Carnitine Palmitoyltransferase 1A Expression and Improves Fatty Liver

To the Editor:

We read with great interest the article by Orellana-Gavalda et al. about the ameliorating effects of long-term hepatic gene transfer of carnitine palmitoyltransferase 1A (*CPT1A*) on obesity-induced hepatic steatosis, diabetes, and insulin resistance.[1] The authors observed increased lipid oxidation mediated by a significant up-regulation of liver *CPT1A* messenger RNA (mRNA). This effect not only improved lipid and glucose metabolism, but also had direct impact on liver inflammatory stress triggered by high-fat diet (HFD) feeding.

Orellana-Gavalda et al. suggest that increasing hepatic *CPT1A* expression is a valid *in vivo* strategy to reduce obesity-related complications. In a rat model of nonalcoholic fatty liver disease (NAFLD), we observed fairly similar results with indomethacin, a dual pharmacological inhibitor of cyclooxygenase 1 (COX1) (prostaglandin H synthase 1 [PTGS1]) and COX2 (PTGS2). We evaluated the effect of the drug on reversing fatty liver, and we also explored the impact on liver mRNA expression of several lipogenic and glucogenic genes, and nuclear receptors. Rats were given a HFD[2] for 8 weeks, and after this period, animals were randomly divided into two groups. For 4 weeks, along with access to HFD, one group received indomethacin (n = 5 rats, 1 mg/day) every 24 hours, and the second group (n = 5 rats) was fed with HFD; a control group (6 rats) was fed with standard chow diet (SCD) for 12 weeks. We observed that indomethacin significantly revert fatty liver disease (Fig. 1). The most remarkable effects of COX inhibition by indomethacin in the HFD group in comparison with the SCD group were: a 230% increase of liver expression of *CPTA1* mRNA, a 100% increase of liver abundance of *PCK1* mRNA (phosphoenolpyruvate carboxykinase, the main control point for the regulation of gluconeogenesis), and an increase of 84% of *PPARα* mRNA

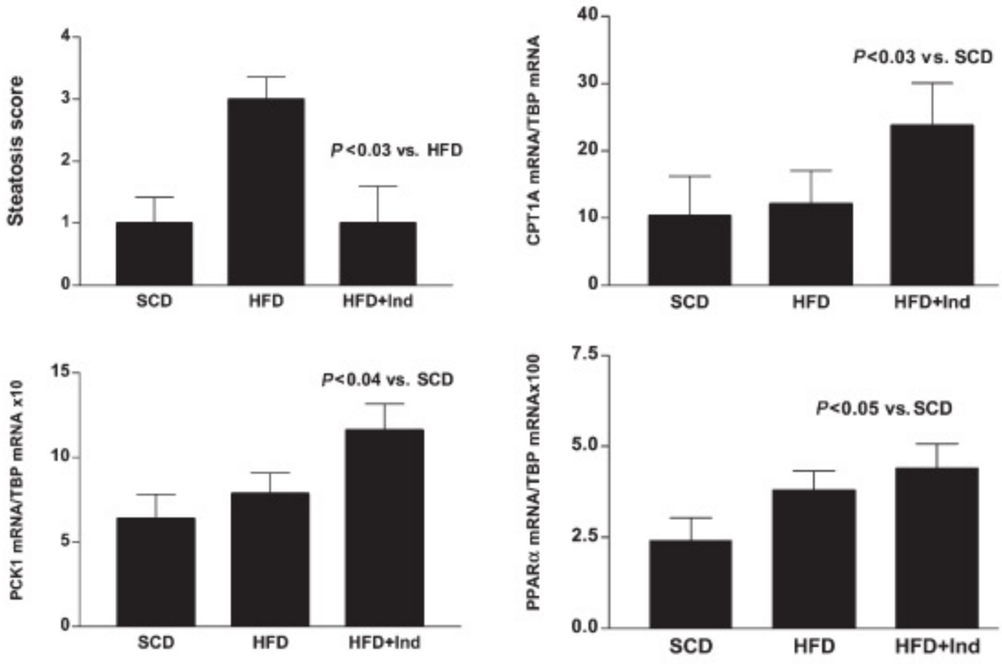

Fig. 1. Liver histology score and abundance of liver CPT1A, PCK1, and PPARα mRNA analyzed by quantitative real-time polymerase chain reaction in each experimental group. Quantitative evaluation of steatosis score from hematoxylin and eosin and osmium tetroxide stain of liver sections at the end of the experiment in all rats from each experimental group. Data are presented as mean ± standard error (SE). For testing steatosis gradation (as a categorical response variable) differences, we used a model with ordinal multinomial distribution and probit as a link function adjusted by body weight as a continuous predictor variable. Liver mRNA expression: Each bar represents mean ± SE of values. HFD, high-fat diet; HFD+Ind, high-fat diet plus indomethacin; SCD, standard chow diet. Real-time polymerase chain reaction was performed for quantitative assessment of mRNA expression. In each sample, gene expression was normalized by the expression of the housekeeping TATA box binding protein (TBP) gene. The ratio was log-transformed and analyzed using analysis of variance.

(peroxisome proliferator-activated receptor alpha, a transcription factor that controls the expression of genes encoding fatty acid oxidation enzymes and mitochondrial fatty acid oxidation) (Fig. 1).

As far as we know, we show for the first time that indomethacin is able to increase liver *CPT1A* mRNA. We can not explain the exact mechanism by which the drug influences liver *CPT1A* expression, although an inhibitory effect of a COX product on the gene expression is an obvious option, but we agree with Orellana-Gavalda et al. that liver *CPT1A* is a prime target to increase beta-oxidation of hepatic long-chain fatty acids. Other explanations are probable. Indomethacin was regarded as a dual PPARγ/PPARα ligand.[3] In addition, the 5′-flanking region of COX2 has several potential transcription regulatory sequences, including CCAAT/enhancer binding protein motif (a gene that specifically regulates hepatic gluconeogenesis and lipogenesis[4]) and two nuclear factor-κB sites (a key modulator of liver injury in NAFLD). Hence, these observations may explain the beneficial effects of indomethacin on NAFLD. In summary, our results represent proof of principle that pharmacological COX inhibition may provide a novel approach for reversing fatty liver by modulating the liver *CPT1A* mRNA expression. These results also add some clues about the potential role of the inducible COX2 and its proinflammatory prostaglandin products in metabolic disorders, including NAFLD.

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