suggest that assessment of regional brain volume changes using voxel-based morphometry (VBM) would provide additional evidence available from our existing MR data as to the pathophysiological significance of brain water and low grade cerebral oedema in HE. In response to these comments, we have revisited the structural scans acquired during our study and performed a post hoc volumetric analysis of the brain in our patient group by three different methods.

We compared the baseline 3D T1 weighted volumetric images (TR/TE 9.6/4.6 ms, flip angle 8°, isotropic 1 mm resolution) against the post amino acid challenge images using VBM (SPM8) [3] for all subjects in a group analysis to identify any areas of white and gray matter volume change. As an alternative measure, we also compared individual subject data using the SIENA approach (Structural Image Evaluation, using Normalisation, of Atrophy, Version 2.2) [4], which provides a measurement of the change in total brain volume between baseline and following the challenge. Finally, we directly subtracted the co-registered baseline and post challenge images for each subject. Images were then visual inspected for evidence of volume change around the brain–CSF boundary [5].

VBM analysis showed no statistically significant areas of volume change in gray or white matter following the amino acid challenge. Likewise, subtraction of co-registered images did not reveal any visual evidence of shifts in the brain size. Formal assessment of boundary shifts using SIENA showed a mean (± SD) difference in total brain volume of –0.2 (± 0.69) percent across all subjects, however the SIENA method is accurate to around 0.2% brain volume change error [4], therefore these results were deemed insignificant.

The results from this paired volumetric analysis showed no significant change in brain volume following the challenge. This is consistent with the original findings of our paper in which we concluded that ammonia can directly drive changes in brain water distribution, suggesting that glial swelling and the redistribution of extra-intracellular water during hyperammonaemia are the likely mechanism of cerebral oedema development in patients with HE. Our findings do not support a vasogenic mechanism for the transient changes in brain water diffusion seen following transient, induced hyperammonaemia in this subject group.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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C reactive protein levels in non-alcoholic fatty liver disease

To the Editor:

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. It represents a wide spectrum of conditions ranging from fatty liver, which in general follows a benign, non-progressive clinical course, to nonalcoholic steatohepatitis (NASH), a more serious form of NAFLD that may progress to cirrhosis and end-stage liver disease. Accurate evaluation of liver fibrosis in patients with NAFLD is important to identify patients who may develop complications. Over time, several biological markers have been studied for evaluating the extent of steatosis, the presence of necroinflammation, and the development of fibrosis to avoid performing liver biopsy, an invasive procedure that still represents the gold standard of diagnosis. The most important parameter to be identified through non-invasive methods is inflammation, as it plays a central role in NAFLD progression. Several biomarkers of inflammation were extensively studied in relation to fatty liver disease. The C reactive protein (CRP) is an acute-phase reactant produced by the liver and has an increased serum concentration in a variety of inflammatory conditions. The assessment of circulating levels of high sensitive CRP (hsCRP) proved to be useful in differentiating between simple steatosis and NASH. Moreover, it seems that high concentrations of hsCRP are associated with extensive liver fibrosis in NASH [1].

In a recent issue of the *Journal of Hepatology*, Zimmermann *et al.* reported the association of hsCRP with various features of NAFLD in a large obese population. The authors concluded that, based on their results, hsCRP may be a marker of steatosis, but not of severity of NAFLD in these patients [2]. The findings of this report are interesting and contribute to our understanding of this issue. However, we have some concerns about the data presented by the authors.

Firstly, circulating hsCRP levels have been reported to be elevated in subjects with diabetes mellitus (DM) and also prediabetes [3,4]. Although it was stated in the article that some of the

patients were diabetic at baseline, there is no information regarding the glucose tolerance status of the other subjects. It is well known that NAFLD is strongly associated with obesity, hypertension, dyslipidemia and also glucose tolerance abnormalities [5]. In addition, all these metabolic problems are risk factors for DM and also prediabetes. In light of these data, we think that some of the study participants may still have overt glucose dysregulation or DM without implementation of the glucose tolerance test. Secondly, there is no data about the blood pressure and lipid profiles of the study participants. A large body of evidence shows that circulating levels of hsCRP differ according to the degree of lipid disorders [6]. This point is also true for hypertension or elevated blood pressure [7]. On the other hand, metabolic syndrome is also strongly associated with circulating hsCRP levels [8]. Lastly, no information about the medications could be seen in the text. We know that hsCRP levels are easily affected from the medications started for the metabolic problems mentioned above [9,10]. Collectively, all these points raise several questions warranting discussion.

We conclude that, before making certain interpretations, presence of major confounders raises some questions about the data presented. In such a case, statistical correlations may also be misleading. It would be appreciated if the authors could present some more data adjusted for topics mentioned above. This could provide the readers of the *Journal* clearer information regarding the relationship of hsCRP with this clinically relevant condition.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "C-reactive protein levels in non-alcoholic fatty liver disease"

To the Editor:

We thank Dr. Dogru *et al.* for their comments on our paper on C-reactive protein (CRP) levels in relation to various features of non-alcoholic fatty liver disease (NAFLD) among obese patients [1]. In our study of 627 obese adults with liver histology and measures of body mass index (BMI, kg/m^2), and high sensitivity (hs)-CRP, we confirmed a strong association between BMI and hs-CRP, with a 20% increase in hs-CRP level per 10% increase in BMI. Also, we observed a positive association between hs-CRP and liver steatosis, but no BMI independent association between hs-CRP and non-alcoholic steato-hepatitis (NASH), hence questioning whether CRP can be used as a marker of severity of NAFLD [1].

We fully acknowledge that circulating hs-CRP levels may not only associate with obesity, but also with pre-diabetes and diabetes [2,3], which in turn may associate with NAFLD [4]. However, obesity (which was adjusted for by use of BMI) is also strongly associated with both diabetes and NAFLD [5]. Therefore, further adjustment for diabetes or glucose tolerance abnormalities would not be expected to change our results notably. The same argument holds for the effect of further adjustment for hypertension and lipid profiles. Still, to meet the concern of Dr. Dogru *et al.*, we have reassessed the mentioned metabolic factors in two of the study cohorts. We can now report that in the OBBO cohort, hs-CRP was associated with triglycerides ($p = 0.02$), but not with glucose tolerance status (test across categories of normal glucose tolerance, impaired fasting glucose [IFG], impaired glucose tolerance [IGT], combined IFG-IGT, and diabetes), systolic or diastolic blood pressure, total cholesterol, HDL-cholesterol, or LDL-cholesterol. In the Nice Obese Subjects cohort, we observed a negative association between CRP and high blood pressure ($p = 0.002$), but no association between CRP and, respectively, diabetes, glucose intolerance, triglyceride levels, HDL-cholesterol, or the metabolic syndrome. Hence, adjustment for triglycerides and blood pressure could be considered, but would, due to the association with BMI, not be expected to change conclusions.

An additional concern raised by Dr. Dogru *et al.* was the effect of medications on the observed associations. A reduction in hs-CRP caused by medications given primarily to patients with severe metabolic disturbances and related severe NAFLD, could hypothetically explain our finding of no association between hs-CRP levels and severity of NAFLD. Our reassessment of the OBBO cohort revealed that hs-CRP was not associated with