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Re : Manuscript number 31513

Dear Sirs,
We thank the reviewers and editorial board for their critique of our manuscript 'Changes in human hepatic metabolism in steatosis and cirrhosis'. Below we address all the points raised by the reviewers in order of appearance and we have amended the text in our revised document to reflect their suggestions. We include a version of the manuscript with changes in red for clarity. We have also supplied a final version of the manuscript with tracked changes incorporated.

Reviewer 503530 : I think that there are few reports of the analysis by the NMR. There are some questions. Is the NMR superior to other assays including the HPLC? It is the analysis of the liver tissue, how do you reflect it in blood? Is it inspection by liver biopsy every time? If liver biopsy is necessary, I think that the diagnosis is easy histologically.

This referee correctly notes that there are indeed few reports describing a detailed NMR-based metabolomics assessment of human liver tissue with most studies either considering rodent models or human serum alone. We have chosen to use NMR as a consequence of its power for unbiased detection of endogenous and exogenous compounds in our complex liver digests. In terms of superiority compared to HPLC, one obvious advantage is the relatively efficient sample preparation and processing time. Our samples were rapidly processed from snap frozen tissue and there is no need for derivatization, use of large volumes of solvents or column equilibration as required for HPLC. Importantly given the exploratory nature of our study, NMR also has the advantage of providing structural identification of unknown components in our samples. Furthermore our strategy is non selective, permitting detection of all appropriately sized compounds at sufficient concentration in the sample and used only a very small volume of sample for analysis. However it is worth commenting that it is possible to couple HPLC with subsequent NMR, for example to preconcentrate samples and aid sensitive assessment of less abundant compounds. We have added text to our discussion section in light of this comment.

The referee also questions how hepatic metabolic signatures might be reflected in blood and if biopsy is necessary since diagnosis is easy histologically. Here we have utilized tissue samples collected during the transplantation procedure (both donor and patient diseased tissue). This has allowed us to identify metabolic species that distinguish disease states and are hepatic in origin. However we acknowledge that whilst it is indeed easy to diagnose patients with a biopsy, our ultimate aim is to utilize less invasive

serological analysis that could yield diagnostic and prognostic information in future studies. Thus we would now move to seeing whether out unknown metabolites are present in serum. There is good evidence in the literature to suggest that women with metabolic disturbance associated with polycystic ovary syndrome (that also associates with NAFLD) show altered serum amino acid and carbohydrate metabolites and thus our suggestion is reasonable. We have added text to the discussion to highlight this.

Reviewer 70280 : This is an interesting study on an important topic. The authors should be congratulated.

We are pleased that this referee acknowledges that this is an important study that sheds light on the mechanisms of human disease.

Reviewer 58872 : This Authors should at large discuss the following issues concerning exercise and quote the related articles: Recent results indicate that mitochondrial UCP3 activity affects metabolism well beyond fatty acid oxidation, regulating biochemical pathways associated with amino acid metabolism and redox status. That select metabolites were altered in liver of UCP3 Tg mice highlights that changes in muscle UCP3 activity can also affect other organ systems, presumably through changes in systemic metabolite trafficking, as evident in.....FASEB J. 2016 Nov 10. pii: fj.201600914R. [Epub ahead of print] A novel amino acid and metabolomics signature in mice overexpressing muscle uncoupling protein 3 and..... Have guidelines addressing physical activity been established in nonalcoholic fatty liver disease? World J Gastroenterol. 2012 Dec 14;18(46):6790-800. doi: 10.3748/wjg.v18.i46.6790

This referee is correct to highlight the importance of exercise. Our steatotic donor material was collected from tissue rejected for transplantation and where the exercise history of the donor was unknown. All our patients with NAFLD are treated according to standard NICE guidelines, which incorporate lifestyle modifications linked to improving diet and increasing exercise levels. Those patients who do not respond to this approach are then offered pharmacological treatment including statins and particularly pioglitazone for those with advanced fibrosis. Patients with metabolic syndrome may also be offered insulin sensitizers such as metformin. However it is important to note that our patients with advanced cirrhosis in the NASH and ARLD groups can exhibit significant sarcopenia and reduced BMI so find physical activity challenging. Thus it would be hard to interpret whether variations in physical exercise can account for all the metabolic changes we see between our patient groups. We have added text to the discussion section to highlight these comments.

It is indeed correct to note however that systemic metabolism in muscle for example has an effect on hepatic activity. This could be through altered consumption or release of fatty acids or glutathione by muscle or as a consequence of enhanced oxidative stress in exercising muscle. The reviewer has suggested we should discuss the potential contribution of skeletal muscle UCP3 to this effect. A few studies have linked expression of variant forms of UCP3 to development of NAFLD in humans with increased expression in skeletal muscle related to increased metabolic rate and lower BMI. In contrast earlier studies using the obese zucker rat model suggest that effects of obesity and exercise lead to decreased UCP3 levels in some muscles and not others. However in the study cited by the reviewer (Aguer et al FASEB, Epub) it is important to note that comparisons were made between mice that had normal or overexpressed levels of UCP3 in skeletal muscle but genetic variants were not considered. Thus it is currently hard to interpret what the contribution of muscle UCP3 to human hepatic metabolism in our patient groups may be. To our knowledge no one has yet looked in cirrhosis with or without sarcopenia in humans. Variations between the NASH and ARLD groups (both of whom may have varying levels of sarcopenia) however suggest that sarcopenia alone cannot explain our patterns of metabolites. We have added some text to the discussion section in light of this reviewers suggestion.We thank the reviewers for their valuable suggestions that have significantly improved our manuscript. We hope that the revised version will be deemed suitable for publication in the Journal of Gastroenterology.

Sincerely

Dr Patricia Lalor (on behalf of the authors)