**Author's response to reviews**

**Title:** 1H NMR-based Metabonomic profile of Rats with Experimental Acute Pancreatitis

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**Author's response to reviews:** see over
Dear editor:

We have revised our manuscript according to the reviewers’ comments. The main concerns are addressed as following:

1. About the effectiveness of sodium taurocholate treatment in inducing acute pancreatitis.

   Answer: Retrograde pancreaticobiliary duct injection with sodium taurocholate is a common and classic method to induce acute pancreatitis all over the world and we have successfully applied this method in our previous studies, relevant reference was mentioned in the manuscript:


2. About some inconsistencies in our results and present literatures.

   Answer: 3-HB is a ketone body synthesized by acetyl-CoA in liver as an energy source utilized by brain under the condition of hypoglycemia. Acetyl-CoA is the product of $\beta$-oxidation of fatty acid. In the early phase of acute pancreatitis, due to acute systemic inflammation response
syndrome, acute pancreatitis always triggers a hypercatabolic state and disorder in lipid metabolism, abundant fatty acid were mobilized for energy supply. As a main product of oxidation of fatty acid, 3-HB is elevated logically with the catabolism of fatty acid. In reference 32, the authors observed a decrease in 3-HB, but they did not explain the reason why it decreased.

Lactate is mainly generated by anaerobic glycolysis and not the main way for energy supply in normal condition. In the condition of intense workout or hypoxia, insufficient oxygen supply lead to restrained Krebs circle reaction, less ATP production, enhanced glycolysis, decreased removal ability of liver and kidney and ultimately accumulation of plasma lactate[24, 25]. In the early stage of acute pancreatitis, due to the increased energy consumption and oxygen deficit, anaerobic glycolysis was enhanced, and then resulted in raised level of lactate. In clinical, patients with acute pancreatitis on admission usually exhibit symptoms of hypovolemia, oxygen deficit and high energy consumption. Their arterial blood gas analysis usually suggest hemoconcentration, low oxygen partial pressure and elevated lactate, those changes are more obvious in severe cases. Elevated lactate can often be restored after oxygen inhalation and fluid resuscitation. In reference 27, the patients were mild cases and there is no mention about the time of obtaining the urinary samples. But it is the time that could obviously influence the lactate level because there would be quite difference before and after fluid resuscitation and oxygen inhalation. In our experiment, the blood samples were obtained from rats with experimental acute pancreatitis, there was no intervention before obtaining the samples. So our results was the real reflect of the model and it should be more reliable.

3. About the discussion.

Answer: This section have been revised according to the reviewers’ advice.
4. About the meaning of investigating the metabolic profiles.

Answer: Acute pancreatitis is a common inflammatory disease of the pancreas with serious metabolic disturbance. But the specific metabolic process of this disease is still unclear and there is no specific therapy for this disease. Detection of characteristic metabolite changes in AP might increase our understanding of the pathophysiology of the disease, allow early diagnosis, screen the biomarkers for the severity classification and identify potential therapeutic targets. That is the meaning of investigating the metabolic profiles.

Early diagnosis will allow timely intervention and better prognosis. Although serum amylase are commonly used for the diagnosis of acute pancreatitis, they are restrained in several occasions. Amylase is limited in sensitivity, specificity, and positive and negative predictive value, and serum amylase alone cannot be used reliably for the diagnosis of acute pancreatitis. It may remain within the normal range on admission in as many as one-fifth of patients. Further, Serum amylase concentrations may be normal in alcohol-induced acute pancreatitis and hypertriglyceridemia. Also, serum amylase concentrations might be high in the absence of acute pancreatitis in macroamylasaemia, in diseases of the salivary glands and in extrapancreatic abdominal diseases associated with inflammation, including acute appendicitis, cholecystitis, intestinal obstruction or ischemia, peptic ulcer, and gynecological diseases. (references: 1.Gomez D, Addison A, De Rosa A, Brooks A, Cameron IC. Retrospective study of patients with acute pancreatitis: is serum amylase still required? BMJ Open 2012 Sep 21;2(5). 2. Scott Tenner, John Baillie, John DeWitt, Santhi Swaroop Vege: American College of Gastroenterology Guidelines: Management of Acute Pancreatitis. Am J Gastroenterol 2013; doi: 10.1038/ajg.2013.218) Thus, serum amylase may not best for the diagnosis of acute pancreatitis, metabolic biomarkers may help the diagnosis of AP
more accurate.

5. Why we consider these parameters are helpful for investigating the pathogenesis of AP or identifying potential therapeutic targets? Whether the changed metabolic profile correlated with the severity of AP?

Answer: Patients with acute pancreatitis always exhibit metabolic disturbances. Due to acute systemic inflammation response syndrome, AP always triggers a hypercatabolic state through mobilizing of multiple metabolic pathways. The discrepancy metabolites we detected were involved in these metabolic pathways. We could retrace the genesis process in vivo and find the key factors which affect the development of the disease. These factors will be regarded as critical biomarker for the study of physiopathological mechanisms as well as important therapeutic targets of acute pancreatitis.

In the present study, we just designed to investigate the metabolic changes in AP. We did not focus on whether the changed metabolic profile correlated with the severity of AP. From the preliminary results, we have decided to conduct an experiment to explore whether the changed metabolic profile correlated with the severity of AP.

6. What is the reason that the 12 hours is chose? What about the biomarkers levels in an even earlier stage of AP?

Acute pancreatitis is a dynamic pathophysiological process. According to the literature, time-course changes existed in experimental acute pancreatitis models. The time of progression to the peak of inflammatory response varied according to different models. For example, in CCK-8-induced models with acute pancreatitis, the pancreatic weight/body weight ratio (pw/bw, quantification of the pancreas edema), amylase and IL-6 reached the maximum levels at 4 h after
inducing acute pancreatitis. However, in the biliary type of acute pancreatitis, the ratio pw/bw reached the maximum level at 48 h, IL-6 reached its maximum level at 16 h. Here, we selected the retrograde pancreaticobiliary duct injection with sodium taurocholate to induce acute pancreatitis. Another study showed that total TNF-α levels in serum remained increased up to 9 h post-induction of AP through retrograde pancreaticobiliary duct injection with sodium taurocholate. Additionally, there is report about the changes in microelement in pancreatitis, and significant difference was detected 12h. So, in this study, we selected 12h to obtain the blood samples. (References: 1. Takács T, Farkas G Jr, Czakó L, Jármay K, Mándi Y, Lonovics J: Time-course changes in serum cytokine levels in two experimental acute pancreatitis models in rats. Res Exp Med (Berl).1996;196(3):153-61. 2. Granell S. Pereda J. Gomez-Cambronero L. Cassinello N. Sabater L. Closa D. Sastre J: Circulating TNF-alpha and its soluble receptors during experimental acute pancreatitis. Cytokine 2004, 25(4):187-91. 3. Dabrowski A, Bojarski K, Maciejewski R.:Magnesium homeostasis disorders caused by experimental acute pancreatitis. Med Sci Monit 2003, 9(5):BR183-7.)

In the present study, we just attempted to investigate whether there were any metabolic changes in acute pancreatitis with selecting only one time point---12h. From the preliminary results, we have set about to investigate the dynamic process of metabolic changes which will disclose an metabolic profile in an even earlier and later stage of acute pancreatitis.

7. The metabolic disturbance detected in the AP group may not be specific for AP. It is possible that the changed metabolic profile is caused by excessive inflammation rather than pancreatitis? Answer: The conception that AP could cause metabolic disturbance is well known. AP causes severe systemic inflammatory response syndrome is also well known. The metabolic changes
might be caused by excessive inflammation, but the excessive inflammation here is caused only
by AP. AP is the initial and only reason here to contribute to the metabolic changes. So, it is proper
to regard these changes to be specific for AP, at least in this experiment, it is.

8. Other spelling mistakes have been revised. We deleted “a controlled experiment ” in the title.
The “Compared to sham operation group” in the table has been described in the footnote. All
changes made in the revised manuscript have been highlighted in red.

Best wishes

Yours

Juan Li