Reply

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We thank Sookoian et al. for the interesting comments on our article.1 However, it must be said they have downplayed the novelty of our results that rely not in the increase in BCAA, but rather in the importance of other amino acids (AA), like glutamate (GLUT), serine (SER), glycine (GLY), and the new GSG index, calculated as GLUT/(SER + GLY), that we found able to well discriminate liver fibrosis F02 from F34.

We agree with them that the use of GLUT as a biomarker of altered liver metabolism has not been considered enough, although several studies have provided evidences that GLUT is increased in metabolic diseases (table 3, Gaggini et al.1). GLUT participates in several metabolic reactions, from synthesis of glutathione (GSH) and SER to transamination of alanine and as substrate of the TCA cycle (Fig. 1). Increased ALT has been used as a surrogate marker of nonalcoholic fatty liver disease (NAFLD), although not all NAFLDs have increased AST and ALT. This is not surprising given that these are intracellular enzymes released in plasma only when there is cellular damage, whereas GGT is mainly expressed on the cell surface (Fig. 1).

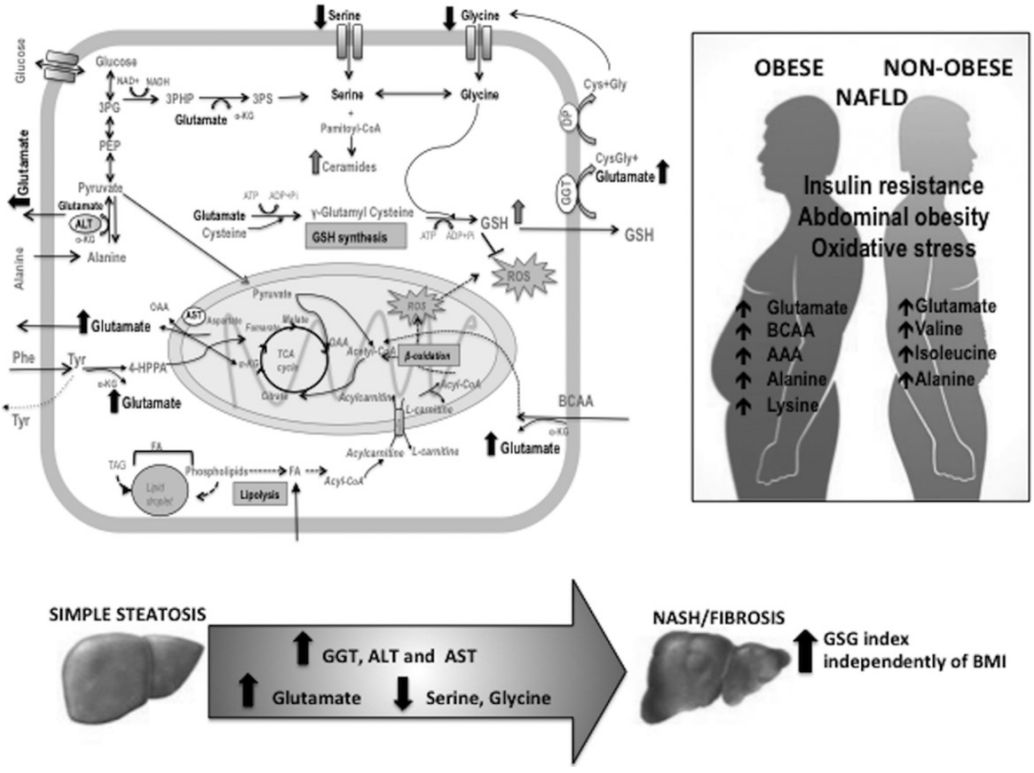

Figure 1

Imbalance of circulating amino acids and transamination reactions in NAFLD with/without obesity. The left panel shows the hepatic metabolic pathways in which amino acids (in particular the components of the GSG index glutamate, serine, and glycine) are involved. Mitochondrial oxidative stress leads to increased production of reactive oxygen species (ROS) that, in turn, stimulates the synthesis of glutathione (GSH) from glutamate, glycine, and cysteine. Once GSH reaches the cell surface, it is transaminated by GGT and glutamate is released into the bloodstream. Glutamate is also produced from transamination by ALT and AST. A characteristics of subjects with NAFLD is the presence of IR (in liver, muscle, and adipose tissue), increased waist circumference (i.e., abdominal obesity), and oxidative stress (see right panel). The serum concentrations of several amino acids are increased in NAFLD, but when the subjects are grouped according to body mass index, only a few (glutamate, alanine, and, of the BCAA, valine and isoleucine, but not leucine) remained increased in nonobese NAFLD, indicating that the IR associated to obesity is a major confounding factor in the association between plasma amino acid concentrations and NAFLD. As NAFLD progresses to NASH and fibrosis, there is an increased release of glutamate and consumption of serine and glycine, reflected by an increase in GSG index (bottom panel). Abbreviations: PEP, phosphoenolpyruvate; 3PG, 3-phosphoglycerate; 3PHP, 3-3-phosphohydroxypyruvate; 3PS, 3-phosphoserine; Cys, cysteine; Gly, glycine; Glut, glutamate; α-KG, α-ketoglutarate; OAA, oxaloacetate; GSH, glutathione; DP, dispeptidase; GGT, gamma glutamyl transferase; ALT or GPT, alanine amino transferase; AST or GOT, aspartate amino transferase; FA, fatty acid; TAG, triacylglycerols; Phe, phenylalanine; Tyr, tyrosine; 4-HPPA, 4-hydroxyphenylpyruvic acid; BCAA = leucine, valine and isoleucine.

Among plasma AA, GLUT showed the highest concentrations in NAFLD versus controls (tables 3-5), a strong association with the degree of liver fibrosis and presence of ballooning (tables 4 and 5).[1] Also Sookoian et al. have found GLUT increased and associated with obesity, dyslipidemia, and dysglycemia. However, most AA concentrations are increased in obese subjects because of insulin resistance (IR); this is not the case of GLUT and GSG index that mark presence of severe liver fibrosis even in nonobese NAFLD.[1]

In severe NAFLD, GLUT is produced by transamination by all liver enzymes, in particular by GGT through degradation of GSH; SER and GLY are used either to synthesize GSH or to form toxic lipids like sphingolipids and ceramides or oxidized. It is also possible that SER might be used to produce alanine,[1] but the activity of this enzyme seems quite low in humans. We proposed that, in subjects with severe NAFLD, an increased GSG index reflects the metabolic status associated with liver fibrosis. Sookoian et al. suggested to use the plasma SER/GLUT ratio as a marker of disease.[1] We explored this ratio in our data set, finding that, although it was decreased in NAFLD, compared to the GSG it could not discriminate F02 from F34 and had a weaker association with IR.

In conclusion, in NAFLD, glutamate metabolism is increased independently of obesity, strictly linked to mitochondrial oxidative stress and synthesis of toxic lipids. The new GSG index reflects this metabolic imbalance and might help in discriminating severity of liver disease even in nonobese NAFLD.

References

1 Gaggini M, Carli F, Rosso C, Buzzigoli E, Marietti M, Della Latta V, Ciociaro D, et al. Altered amino acid concentrations in NAFLD: impact of obesity and insulin resistance. *Hepatology* 2017 Aug 12. doi: 10.1002/hep.29465. [Epub ahead of print]