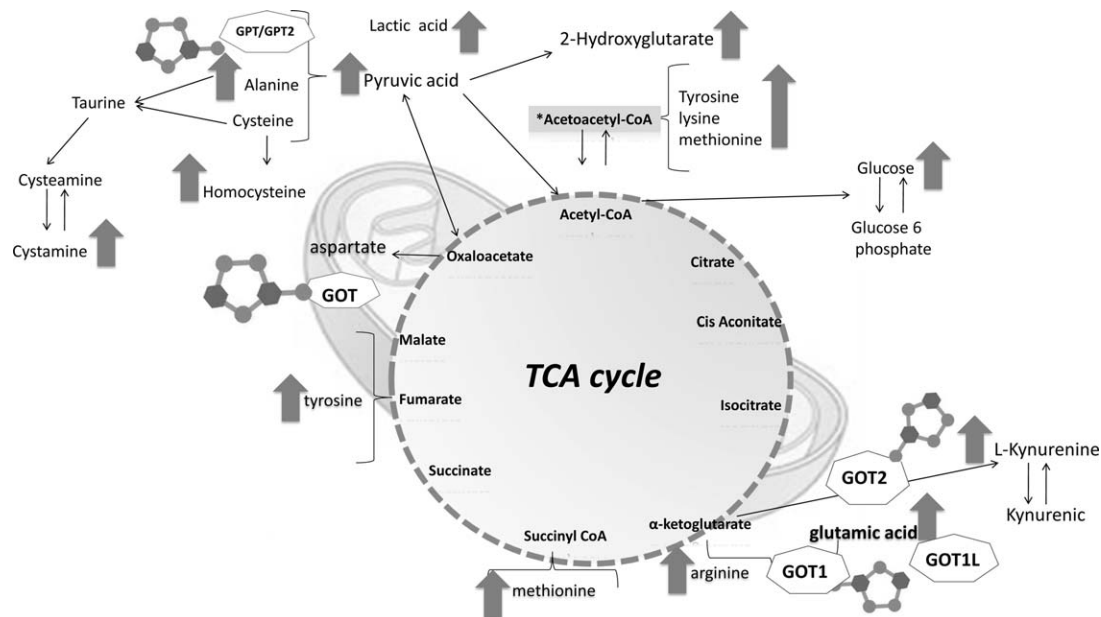


# The Nonalcoholic Steatohepatitis Metabotype: Imbalance of Circulating Amino Acids and Transamination Reactions Reflect Impaired Mitochondrial Function

## TO THE EDITOR:

We read with interest the article of Gaggini et al. reporting plasma concentrations of amino acids (AAs) in nonalcoholic fatty liver disease (NAFLD).<sup>(1)</sup> The researchers observed that patients with NAFLD had high levels of isoleucine and valine (branched-chain amino acids; BCAA), tyrosine, alanine, lysine, and glutamate. More specifically, the researchers found that BCAA correlated with alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels; therefore, they concluded that plasma levels of amino-

transferases reflect increased transamination reactions, and that amino acids are markers of hepatic inflammation and fibrosis. Although these results are confirmatory findings of knowledge that is already known and of which the associated molecular mechanisms have been extensively studied,<sup>(2)</sup> some points deserve to be highlighted. For example, we observed that in patients with NAFLD, circulating levels of some essential AAs, L-glutamic acid, and other metabolites (2-hydroxyglutarate), were significantly associated not only with serum levels of AST, but also liver transcriptional activity and protein level of aminotransferases<sup>(2)</sup>



**FIG. 1.** Imbalance of circulating amino acids and transamination reactions in NAFLD. Figure depicts circulating metabolites up-regulated in biopsy-proven NAFLD and the levels of which are significantly associated with liver expression of transaminases (ALT and AST).<sup>(2)</sup> Upward pointing arrows highlight up-regulated levels of gluconeogenic amino acids (alanine, cysteine, methionine, aspartate, arginine, and glutamic acid) and gluconeogenic or ketogenic amino acids (tyrosine and lysine, respectively).<sup>(2)</sup> Transamination reactions: **GPT** (Alanine aminotransferase 1, enzyme entry: 2.6.1.2): L-Alanine + Oxoglutaric acid  $\leftrightarrow$  Pyruvic acid + L-Glutamic acid. **GPT2** (Alanine aminotransferase 2, enzyme entry: 2.6.1.2): L-Alanine + Oxoglutaric acid  $\leftrightarrow$  Pyruvic acid + L-Glutamic acid. **GOT1** (Aspartate aminotransferase, cytoplasmic, enzyme entry: 2.6.1.1 and 2.6.1.3): L-Aspartic acid + Oxoglutaric acid  $\leftrightarrow$  Oxalacetic acid + L-Glutamic acid. **GOT2** (Aspartate aminotransferase, mitochondrial, enzyme entry: 2.6.1.1 and EC: 2.6.1.7): L-Aspartic acid + Oxoglutaric acid  $\leftrightarrow$  Oxalacetic acid + L-Glutamic acid. **GOT1L1** (Putative aspartate aminotransferase, cytoplasmic 2, enzyme entry: 2.6.1.1): L-Aspartic acid + Oxoglutaric acid  $\leftrightarrow$  Oxalacetic acid + L-Glutamic acid. \*It must be emphasized that in the skeletal muscle, especially under physiological conditions of starvation and pathological stress responses, BCAA catabolism may be activated to support anaplerosis and acetyl-CoA as a substrate. It is controversial whether this reaction is unique to the skeletal muscle and not other organs.

(Fig. 1). We also found that 2-hydroxyglutaric acid, L-glutamic acid, and alanine/pyruvate ratio were significantly associated with NAFLD disease severity.<sup>(2)</sup> Notably, L-alanine/pyruvate ratio significantly correlated with body mass index even after adjusting for homeostasis model assessment of insulin resistance.<sup>(2)</sup>

Furthermore, cross-sectional and longitudinal data from the Framingham Heart Study showed implicated dysregulated glutamate cycling and AA metabolism in metabolic risk.<sup>(3)</sup> For instance, glutamic acid and 2-ketoglutaric acid were both significantly associated with ALT and AST in discovery (n = 650) and replication (n = 554) analysis.<sup>(3)</sup> Most important, among 119 plasma metabolites assessed, the top AA biomarker was glutamic acid, which was directly associated with obesity, dyslipidemia, and dysglycemia in discovery and replication data sets.<sup>(3)</sup> The ratio serine/glutamic acid, a reflection of serine-pyruvate and alanine transaminase activity, was also significantly associated with metabolic syndrome components.

Interestingly, not only all above-mentioned metabolic reactions occur in the mitochondrial matrix, but each of the resulting metabolite is a by-product of the Krebs cycle (Fig. 1).

Remarkably, transaminations of AAs, including BCAA, are, in part, mediated by AST and ALT (Fig. 1). Likewise, NAFLD severity is associated with impaired mitochondrial (mt) function,<sup>(4,5)</sup> altered mt-morphology,<sup>(4)</sup> and increased mt-genome variability.<sup>(5)</sup> Together, it is plausible to speculate that the circulating “nonalcoholic steatohepatitis (NASH) metabolite” resembles a “mitochondrial disease.”

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## REPLY:

We thank Sookoian et al. for the interesting comments on our article.<sup>(1)</sup> 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.<sup>(1)</sup>). 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