NAFLD

Metabolic make up of NASH: from fat and sugar to amino acids

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NAFLD is regarded unquestionably as one of the components of the metabolic syndrome. Hence, metabolic perturbations occurring in the fatty liver become a systemic metabolic derangement. The phenotypic switching from fatty liver to NASH entails a reprogramming of liver metabolism to fit a stressful metabolic environment.

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NAFLD consists of a spectrum of disease. Once NASH (the most severe form of NAFLD) develops, the systemic effects of metabolic derangement in the liver are more dramatic. An elegant study using genome-scale metabolic modelling has now provided challenging information regarding putative altered enzymatic reactions associated with NASH (Figure 1).[1] The study has also proposed novel alterations of the so-called liver metabotype (that is, the metabolic profile) that include a distinctive amino acid imbalance [Au:OK?] among other metabolites, such as proteoglycans and phosphatidylserine.[1] This strategy takes advantage of a computational liver model named *iHepatocytes2322*, which is a reconstruction of proteomic data focused specifically on hepatocytes that includes all the reactions and metabolic pathways associated with liver cells. This tool was used to further interpret results of differential liver gene expression in patients with NAFLD at different stages of disease severity in a holistic manner. So, what have we learnt from this metabolic modelling and how can we put the results of this study into a clinical context?

The new findings[1] suggest that NASH might be associated with elevated blood concentrations of chondroitin sulphate. This finding is perhaps not surprising because chondroitin sulphate modulates the cellular processes involved in inflammation, including cytokine and growth factor activities, and is a component of the extracellular matrix, which is increased in fibrosis. Notably, a genome-wide association study (GWAS) of patients with NAFLD showed that a gene variant near the *NCAN* locus (neurocan core protein) was associated with NASH.[2] Moreover, Mardinoglu *et al.*[1] predicted that there is an imbalance in amino acid metabolism that brings new players in the pathogenesis of NASH to our attention. Consequently, we should now consider blood levels of fat and sugar, and the levels of branched-chain amino acids (BCAAs) and metabolites of the glutamine-cycling pathway in NASH pathogenesis.[3] Although this metabolic signature has been reported in related clinical conditions, such as obesity,[3] type 2 diabetes mellitus,[4] and cardiovascular disease,[5] these findings reinforce the concept of the potential application of metabolic biomarkers in the clinical setting. The main hypothesis behind these observations is that the increase in BCAAs in NAFLD is associated with perpetuation of the insulin resistance phenotype as these amino acids are primarily gluconeogenic. Taken together, these observations emphasize that NAFLD is not a bystander condition associated with the metabolic syndrome, but is critically involved in its development.[6] Perhaps we can now suppose that all the global metabolic derangements of the metabolic syndrome start in the fatty liver?

The authors also revealed that many of the intermediate metabolites of the tricarboxylic acid cycle (TCA) were shifted toward a 'cellular and mitochondrial stress' phenotype. Concomitantly, they described a downregulation of TCA-related mitochondrial reactions inferred from tissue expression data. Indeed, the predicted downregulation

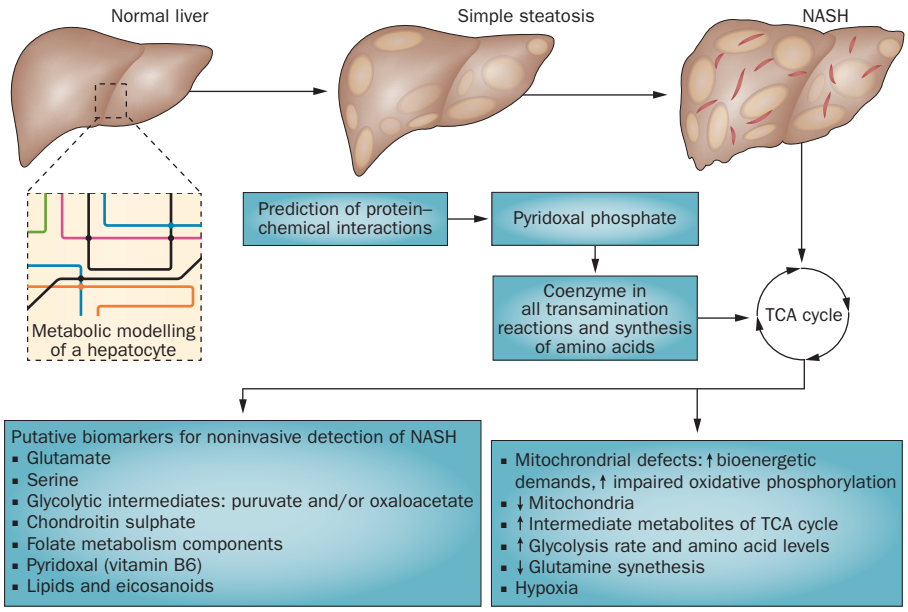

Figure 1 | Proposed hypothesis regarding metabolic changes associated with NAFLD progression and the prediction of protein–chemical interactions based on metabolic modelling. The figure highlights the findings by Mardinoglu *et al*.[1] Overall, liver metabolism in NASH is shifted toward a cellular stress metabolic response with a predicted accumulation of pyruvate and glutamate,[1] suggesting an imbalance between entry and removal of TCA cycle intermediates. Putative 'metabolic biomarkers' might be useful for early disease detection. *In silico* exploration of interactions between drugs and target proteins shows pyridoxal phosphate as highly predicted to be involved. Abbreviation: TCA, tricarboxylic acid. [Au: legend cut owing to space limitations]

of mitochondrial function is not surprising because previous observations have shown that NASH is associated with decreased mitochondrial number,[6] morphological mitochondrial defects[7,8] and downregulation of the transcriptional and protein activity of members of the oxidative phosphorylation chain.[7] Finally, the researchers inferred that NASH is associated with serine deficiency and with an increase in serum glutamate levels. Consequently, they suggested that, in patients with NASH, some enzymatic reactions might either be suppressed (those involved in serine conversion from glycine) or enhanced (those involved in glutamate conversion from α-ketoglutarate and valine, BCAT1). Of note, the increase in glutamate levels reported here and in other metabolic-syndrome-associated conditions is difficult to explain by an increase only in BCAT1 activity along with decreased activity of several other glutamate-forming enzymes.

On the basis of these new observations, we might wonder whether NASH could be regarded as an 'enzymopathy' of hepatocytes. To answer this question, we need more experimental evidence and to investigate other putative biological explanations. For example, patients with NASH might be carriers of gene variants associated with enzyme defects in either nuclear or mitochondrial proteins. These putative enzymatic defects could be triggered by metabolic insults, such as the risk allele G of the rs738403 single nucleotide polymorphism in *PNPLA3*.[9] Interestingly, an increase in phospholipase A2 activity, as a result of PNPLA3, might explain the increase in prostaglandin E2 levels **[Au:OK? Or activity?]** predicted by Mardinoglu *et al.*[1] through the release of arachidonic acid. However, surprisingly, none of the current GWAS on NAFLD[10] have shown statistically significant associations between NASH and any of the enzymes Mardinoglu *et al.*[1] predicted as being putatively involved in NASH. Moreover, a stressful metabolic environment might trigger epigenetic changes in genomic DNA[6] and in the mitochondrial genome,[7] leading to, for instance, changes in gene and protein expression, and thereby enzymatic impairment. As such, NASH can probably be regarded as a consequence of one or more enzymatic defects, but if the condition is a consequence of liver insulin resistance, the underlying causes remain to be established. Notably, alterations in the intermediates of the synthesis of triacylglycerols or phospholipids (such as lysophosphatidyl choline) are now recognized to contribute to altered insulin signalling, and thereby, to NAFLD and/or NASH and insulin resistance.

Despite these interesting results,[1] certain limitations of the findings predicted by systems biology modelling must be highlighted: the main results were not replicated in a well-characterized and powered sample of patients and controls; the transcriptomic data were retrieved from a public repository, thus, relevant clinical and biochemical information were lacking; results from gene expression data do not seem to be adjusted by multiple testing; and direct measurements did not confirm predictions of altered metabolites. From a morphological perspective, one might argue that the proposed model ignores other cell types from the analysis that have a proven profound effect on NASH pathogenesis (such as kupffer cells or hepatic stellate cells). More importantly, metabolic modelling cannot explore environmental confounders, and this missing information precludes, for instance, the assessment of host–gut microbiota metabolic interactions.

Finally, how can the new findings be translated to the therapeutic field. The authors speculated about amplifying the activity of certain enzymes involved in serine synthesis to restore the diminished serine levels in NASH. Despite being an attractive approach, this type of strategy, which requires gene therapy or RNA and/or protein editing, is still new, even for the treatment of well-known inherited metabolic disorders. Likewise, the authors proposed blocking the enzymatic activity of enzymes that drive increased levels of glutamate (BCAT1). These strategies might well have a place in the treatment of NAFLD—these interventions are reminiscent of chemotherapeutic strategies used in cancer (inhibition of glycolysis is considered a powerful tool to restore respiratory abnormalities in the mitochondria of cancer cells)—but are far from the clinic. In fact, less radical, therapeutic alternatives for the treatment of patients with NASH (such as lifestyle intervention) are available or could be developed (dietary supplementation with enzyme cofactors or serine). In summary, future prospective clinical studies are needed to refute or confirm the hypotheses that emerged from the new study.[1] In particular, validation of gene and protein expression data, replication of putative metabolic biomarkers and their clinical predictive power, and thorough experimental assays to prove the potential of enzymatic manipulation as a treatment strategy are required. Meanwhile, we can infer that NASH is the consequence of a whole metabolic injury of hepatocytes that evolves sequentially toward a phenotype of stress response. Any intervention should be implemented before the loss of physiological adaptive mechanisms of the hepatocytes to survival from metabolic stress. With timely intervention, the altered 'metabotype' might be reverted.

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Competing interests
The authors declare no competing interests.

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