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Diabetes and Obesity

THE IMPACT OF ENERGY METABOLISM ON EPIGENETIC MODIFICATIONS RELATED TO THE COMBINATION OF DIABETES AND OBESITY

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1. Fulfilled objectives

1) to identify alterations in metabolic pathways that may predict the impact of obesity and diabetes. We have used high-throughput quantitative analyses and methods for data analysis, mining and computation, and we have combined nested studies and activities; 2) to assess the clinical and metabolomic effect of nutritional measures and bariatric surgery: Patients with successful therapeutic weight loss and diabetes provided the opportunity 3) to explore the effects of reversed metabolic status on the associations between DNA methylation and disease. We have used ex vivo experiments to explore the role of dynamic DNA methylation in metabolic homeostasis.

The multidisciplinary aspect of this project has resulted in multiple results that have been published or are in process of assessment. However, the main findings derived from these objectives include the following:

Abstract

A holistic insight into the relationship between diabetes, obesity and metabolic dysfunction-associated fatty liver disease is an unmet clinical need. Omics investigations can be used to investigate the multifaceted role of altered mitochondrial pathways to promote nonalcoholic steatohepatitis, a major risk factor for liver disease-associated death. There are no specific treatments but remission via surgery might offer an opportunity to examine the signaling processes that govern the complex spectrum of chronic liver diseases observed in extreme obesity. We aim to assess the emerging relationship between metabolism, methylation and liver disease. We tailed the flow of information, before and after steatohepatitis remission, from biochemical, histological, and multi-omics analyses in liver biopsies from patients with extreme obesity and successful bariatric surgery. Functional studies were performed in HepG2 cells and primary hepatocytes. The reversal of hepatic mitochondrial dysfunction and the control of oxidative stress and inflammatory responses revealed the regulatory role of mitogen-activated protein kinases. The reversible metabolic rearrangements leading to steatohepatitis increased the glutaminolysis-induced production of α-ketoglutarate and the hyperactivation of mammalian target of rapamycin complex 1. These changes were crucial for the adenosine monophosphate-activated protein kinase/mammalian target of rapamycin-driven pathways that modulated hepatocyte survival by coordinating apoptosis and autophagy. The signaling activity of α-ketoglutarate and the

associated metabolites also affected methylation-related epigenomic remodeling enzymes. Integrative analysis of hepatic transcriptome signatures and differentially methylated genomic regions distinguished patients with and without steatohepatitis.

Conclusion

We provide evidence supporting the multifaceted potential of the increased glutaminolysis-induced α-ketoglutarate production and the mammalian target of rapamycin complex 1 dysregulation as a conceivable source of the inefficient adaptive responses leading to steatohepatitis.

2. Main results

The combination of diabetes and obesity, which is currently a pandemic, is responsible of multiple clinical associations that deserve careful clinical assessment. We have focused our attention on chronic liver disease because it affects up to 30% of the population worldwide, diagnosis is somewhat difficult and frequently neglected, and pharmacologic treatment is absent. We highlight several main findings: 1. Diabetes, obesity and associated liver disease may be reversed surgically. Changes in lifestyle factors are useful but not as effective. Our findings are related to the systemic regulation of mitochondrial function and oxidative stress. All metabolic comorbidities are mitigated or reversed after successful surgery. As shown in Figure 1, this response is associated with improvement in mitochondrial function and we identify mitochondrial proteins and complex II in oxidative phosphorylation as potentially useful therapeutic targets.

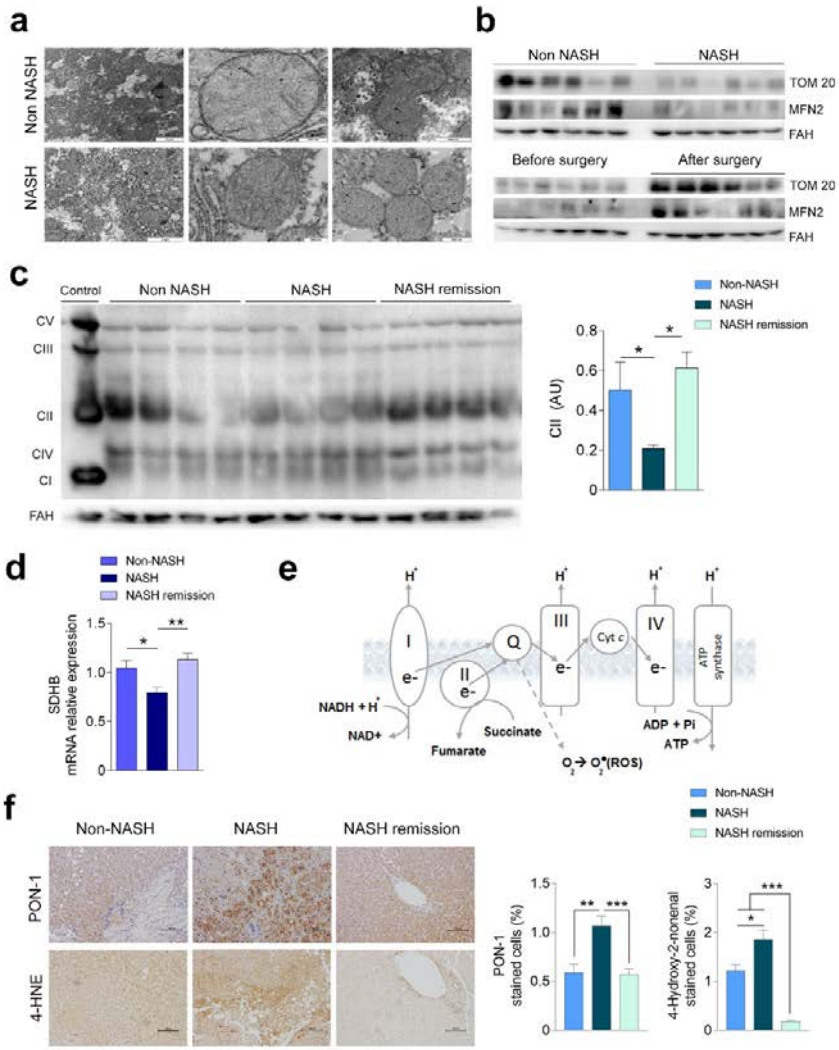

Figure 1. Impairment of oxidative phosphorylation and oxidative stress are reversible in livers of patients with NASH.

(a) Transmission electron microscopy of hepatocyte mitochondria. (b) Representative Western blots of Tom20 and Mfn2. (c) Representative Western blots of the OXPHOS complexes and mean CII quantification (d) mRNA expression of succinate dehydrogenase B recapitulated the changes in complex II (e) A schematic figure representing the importance of complex II in oxidative phosphorylation. (f) Alterations in 4-hydroxy-2-nonenal, and paraoxonase 1 (PON-1). Representative microphotographs (bars indicate 100x magnification) are shown on right, with a quantification of positively-stained area in the left.

Once mitochondrial dysfunction had been identified as a cause or effect of the combination of diabetes and obesity, we explored the consequences in the liver. 2. We found multiple adaptive responses. In the liver, these are mainly associated with

oxidative and inflammatory stresses. Those responses derive in plasma variations in cytokines that may serve as potential predictive markers and therapeutic targets. Explorations of circulating hormones also suggest a systemic effect indicating that multiple tissues and organs are implicated in the combination of diabetes and obesity. 3. Specifically in human liver, we also demonstrated in ex vivo experiments, that the consequences of the disease implicate apoptosis and autophagy (Figure 2). When liver injury is considerable, apoptosis, cell death, increases. More importantly, this effect can be reversed with both surgical and dietary regimens. However, surgical procedures indicated for weight loss is more effective and results may be observed in a short period of time. 4. Unexpectedly, we found that the balance of apoptosis and autophagy appears to be coordinated (Figure 2).

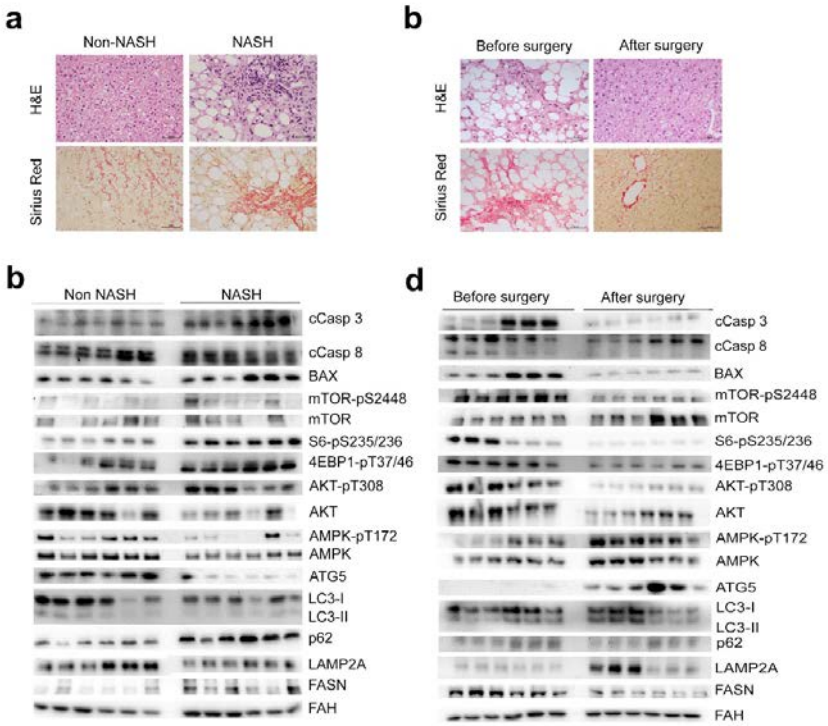

Figure 2. Hepatic AMPK/mTOR-driven pathways coordinate apoptosis and autophagy in liver disease.

(a) Histology differentiated the livers of patients with and without NASH and (b) confirmed the cellular improvement after NASH remission. (c) Representative western blots comparing selected markers in the livers of patients with and without NASH indicated increased apoptosis and compromised autophagy in NASH, accompanied by decreased AMP phosphorylation and increased mTOR phosphorylation. (d) The

same markers examined in patients with NASH before and after surgery indicated that NASH remission reversed apoptosis and reactivated autophagy.

When apoptosis is increased, autophagy is decreased. When apoptosis is reversed, autophagy is also restored. 5. We then performed metabolomic analysis and we found that metabolites in α-KG to succinate conversion and in glutaminolysis distinguished patients with NASH. Those findings indicate that glutamine synthase is a good pharmacological target. 6. Functional studies demonstrated that increased α-ketoglutarate is sufficient to alter mitochondrial metabolism and apoptosis in cultured hepatocytes. Metformin appears to reverse this effect suggesting another potentially useful drug. 7. Microarray analysis of mRNA transcripts identified a hepatic transcriptome signature composed of 345 genes, including 201 downregulated and 144 upregulated genes that distinguished patients with and without NASH. Differences in liver with chronic liver disease appear to be driven by changes in gene expression profiles that enhance glutamine uptake. 8. Altered metabolites are essential in methyltransferase reactions. Measured DNA concentrations of 5-mC were significantly higher in affected livers and genome-wide hepatic DNA methylation screening resulted in largely stable cytosine 5 prime to guanine (CpG) methylation across the livers (Figure 3).

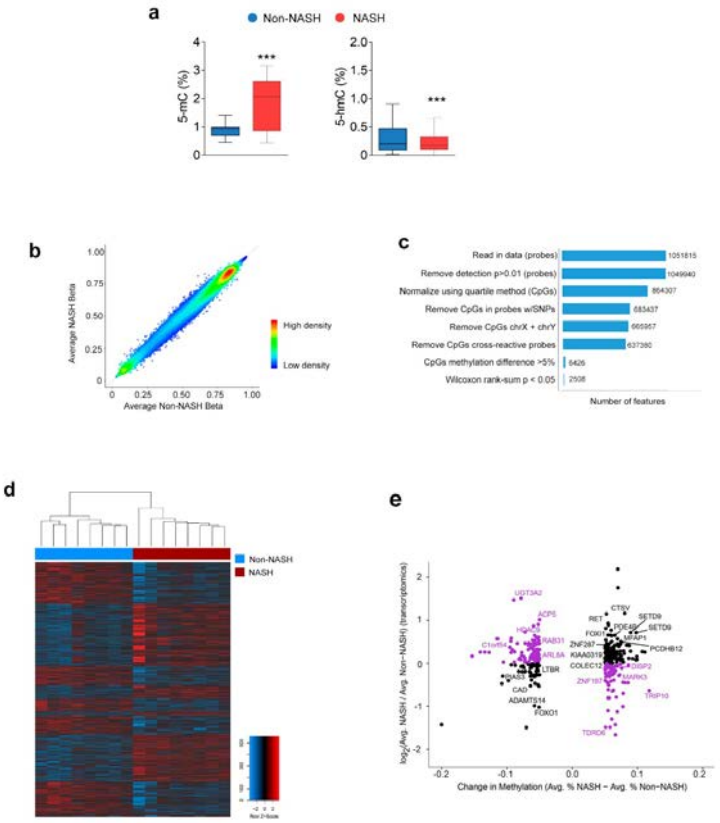

Figure 3. In liver DNA, differentially methylated genomic regions are associated with hepatic gene expression. (a) NASH affected the 5-methylcytosine to 5-hydroxymethylcytosine conversion in genomic DNA with (b) stable bimodal distribution of CpG methylation. (c) There were 2,508 differentially methylated CpGs between groups and (d) unsupervised hierarchical clustering identified a subset of 367 differentially methylated CpGs in promoters that distinguished the livers of patients with and without NASH. (e) The scatter plot shows changes in methylation and gene expression. Purple coloring indicates CpGs in promoters of genes whose expression goes up or down with promoter hypo- or hypermethylation, respectively and labels indicate significant correlations between methylation and gene expression.

We focused on the differentially methylated CpG sites located in promoter regions that correlated with the mRNA microarray data, and we found biologically significant changes associated with 222 hypomethylated and 145 hypermethylated CpG sites (Figure 3).

In conclusion, NASH remission illustrates the need for multiple therapeutic targeting and the importance of mitochondrial dysfunction. The increased

glutaminolysis-induced α-KG production may be a pathogenic candidate to control promotion of the NASH phenotype.

3. Relevant implications attributable to this project

The importance of the combination of diabetes and obesity is not only epidemiological. Current treatments focused on management of glucose metabolism are not sufficiently ambitious. The achieved weight loss is not sufficient to prevent metabolic complications. We also demonstrated that the liver is affected in most patients but diagnosis is poor and treatment is deficient considering the impact on liver mortality associated with diabetes and metabolism. We propose several pharmacological targets that deserve further investigation. Clinically, we demonstrate that usual management based on dietary intervention and drugs with limited effects, usually fails. However, bariatric surgery may rapidly reverse diabetes and its complications. We also propose considering this treatment in patients with metabolically unhealthy combination of diabetes and obesity. Alternatively, our results strongly suggest major efforts in preventing obesity.

4. Publications associated with this project (some still in preparation)

Cabré N, Luciano-Mateo F, Chapski DJ, et al. Glutaminolysis-induced mTORC1 activation drives non-alcoholic steatohepatitis progression. J Hepatol. 2021 May 4:S0168-8278(21)00302-0. doi: 10.1016/j.jhep.2021.04.037. Epub ahead of print. PMID: 33961941.

Hernández-Aguilera A, Casacuberta N, Castañé H, et al. Nonalcoholic Steatohepatitis Modifies Serum Iron-Related Variables in Patients with Morbid Obesity. Biol Trace Elem Res. 2021 Feb 8. doi: 10.1007/s12011-021-02610-8. Epub ahead of print. PMID: 33559024.

Cabré N, Gil M, Amigó N, Luciano-Mateo F, et al. Laparoscopic sleeve gastrectomy alters ^{1}H-NMR-measured lipoprotein and glycoprotein profile in patients with severe

obesity and nonalcoholic fatty liver disease. Sci Rep. 2021 Jan 14;11(1):1343. doi: 10.1038/s41598-020-79485-7. PMID: 33446705; PMCID: PMC7809416.

Cabré N, Luciano-Mateo F, Baiges-Gayà G, et al. Plasma metabolic alterations in patients with severe obesity and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2020 Feb;51(3):374-387. doi: 10.1111/apt.15606. Epub 2019 Dec 11. PMID: 31825539.

Cabré N, Luciano-Mateo F, Fernández-Arroyo S, et al. Laparoscopic sleeve gastrectomy reverses non-alcoholic fatty liver disease modulating oxidative stress and inflammation. Metabolism. 2019 Oct;99:81-89. doi: 10.1016/j.metabol.2019.07.002. Epub 2019 Jul 4. PMID: 31279739.

Castañé H, Baiges-Gaya G, Hernández-Aguilera A, et al. Coupling Machine Learning and Lipidomics as a Tool to Investigate Metabolic Dysfunction-Associated Fatty Liver Disease. A General Overview. Biomolecules. 2021 Mar 22;11(3):473. doi: 10.3390/biom11030473. PMID: 33810079; PMCID: PMC8004861.

Baiges-Gaya G, Fernández-Arroyo S, Luciano-Mateo F, et al. Hepatic metabolic adaptation and adipose tissue expansion are altered in mice with steatohepatitis induced by high-fat high sucrose diet. J Nutr Biochem. 2021 Mar;89:108559. doi: 10.1016/j.jnutbio.2020.108559. Epub 2020 Nov 29. PMID: 33264665.

Luciano-Mateo F, Cabré N, Baiges-Gaya G, et al. Systemic overexpression of C-C motif chemokine ligand 2 promotes metabolic dysregulation and premature death in mice with accelerated aging. Aging (Albany NY). 2020 Oct 26;12(20):20001-20023. doi: 10.18632/aging.104154. Epub 2020 Oct 26. PMID: 33104522; PMCID: PMC7655213.

Luciano-Mateo F, Cabré N, Fernández-Arroyo S, et al. Chemokine C-C motif ligand 2 overexpression drives tissue-specific metabolic responses in the liver and muscle of mice. Sci Rep. 2020 Jul 20;10(1):11954. doi: 10.1038/s41598-020-68769-7. PMID: 32686726; PMCID: PMC7371894.

Hernández-Alvarez MI, Sebastián D, Vives S, et al. Deficient Endoplasmic Reticulum-Mitochondrial Phosphatidylserine Transfer Causes Liver Disease. Cell. 2019 May 2;177(4):881-895.e17. doi: 10.1016/j.cell.2019.04.010. PMID: 31051106.