**Introduction and Objectives:** The microbial communities' control is crucial to maintaining homeostasis of gut-liver axis; clinical evidence demonstrates disruptions of microbiota-gut-liver in individuals with Metabolic-associated fatty liver disease (MAFLD). Foods rich in fiber and polyphenols have been associated with an improvement in microbiota diversity, indexand miRNAs expression. The aim of this study was to evaluate the effect of a supplementation with a mixture of Mexican foods (MexMix): Opuntia ficus indica (nopal), Theobroma cacao (cocoa) and Acheta domesticus (crickets) on gut-liver axis in a MAFLD mice model.

**Materials and Patients:** Thirty C57BL/6J mice were divided into three groups: 1) control: normal diet. 2) HF: high fat diet (60%) and fructose/sucrose water 3) MexMix: HF diet up to week 10, followed by HF diet supplemented with 6.7% nopal, 8.7% cocoa, and 8.7% cricket for 8 weeks.

**Results:** The MexMix animals showed a significantly decreased in body weight, visceral and epididymal fat, adipocyte size, triglycerides, insulin, leptin, and PAI-1; while adiponectin levels increased. Using 16S rRNA gene sequencing, MexMix increased phylogenetic diversity, Firmicutes abundance, and enrichment of 10 beneficial genera, including *Lachnospiraceae*, *Ruminococcaceae*, *Akkermansia*, and *Eubacterium_coprostanoligenes_group*. In the gut, MexMix supplementation significantly increased SCFAs concentration, intestinal crypts depth, *Ocln* and *Cldn1* expression, and decreased *Il6* and *Tnf-a* expression. In liver, MexMix significantly reduced steatosis and Tnfa expression. Besides, MexMix increased nuclear translocation of NFR2 and, in consequence, a higher hepatic expression of *Cat* and *Sod*. MexMix also decreased hepatic expression of miRNA-34a, miRNA-103, and miRNA-33a.

**Conclusions:** Synchronous supplementation with three nutraceuticals, nopal, cacao, and cricket, produced better results compared to previous studies where foods were administered individually. MexMix demonstrated its efficacy as a prebiotic, promoting the growth of beneficial genera and improving intestinal health. These findings indicate that MexMix has the potential to serve as a therapeutic approach for treating MAFLD in patients, as well as other conditions associated with excessive consumption of fats and sugars.

**Ethical statement**

The protocol was registered and approved by the Ethics Committee.

**Declaration of interests**

None

**Funding**

None

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## Effect of methyl donor supplementation on gut microbiota and hepatic expression of key miRNAs in a murine model of MAFLD

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**Introduction and Objectives:** Metabolism-associated fatty liver disease (MAFLD) is the most common liver disease worldwide, and intestinal dysbiosis is associated with its development. Methyl donor supplementation has shown beneficial effects for MAFLD treatment; however, its role on the intestinal microbiota and miRNAs hepatic expression has been poorly studied. The aim of this study was to evaluate the effect of methyl group donor supplementation on gut microbiota and hepatic expression of key miRNAs in a murine model of MAFLD.

**Materials and Patients:** Twenty-four male C57BL/6J mice were divided into three groups: 1) Control: Conventional diet. 2) HF/FS: Diet rich in fats and sugars for 18 weeks. 3) HFMS: HF/FS diet for the first 10 weeks, followed by a HF/FS diet plus orogastric supplementation with methyl group donors for the last 8 weeks.

**Results:** The intestinal microbiota was characterized by 16S rRNA gene sequencing; supplementation with methyl donors modified microbial composition analyzed by beta diversity. In addition, HFMS group strongly tended to increase alpha diversity and induced enrichment of six genus: *Acinetobacter, Anaeroplasma*, *Pseudomonas*, *Stenotrophomonas*, *Tuzzerella*, and *Moraxellaceae* family. HFMS group significantly increased SCFAs fecal concentration and restored intestinal permeability dysfunction by increasing *Ocln* and *Cldn1* expression; consequently, a decrease in liver inflammation was observed due to a decrease in *Tnf-a* expression. On the other hand, HFMS group significantly increased hepatic expression of miR-122 and decreased miR-33a expression.

**Conclusions:** This study offers valuable insights into the role of methyl donors as microbiota modifiers, highlighting their ability to promote restoration of intestinal health and liver metabolism. These findings contribute to the proposition that methyl donors could be a promising strategy for treating MAFLD and hepatic related conditions.

**Ethical statement**

The protocol was registered and approved by the Ethics Committee.

**Declaration of interests**

None

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## Exploring the metabolic and molecular benefits of methyl donor supplementation in a model of metabolic and fatty liver disease

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**Introduction and Objectives:** Metabolic fatty liver disease (MAFLD) is currently the most common cause of chronic liver damage worldwide. Differential methylation in genes and histones has been correlated with metabolic alterations present in the disease. Supplementation with methyl group donor molecules could work as a therapeutic strategy to reverse the progression of the disease.

**Materials and patients:** Male C57BL/6J mice of 20-25g of initial weight were fed with a conventional diet (ND n=8); or a diet high in

fat and sugar (HF n=8) for 18 weeks, or a diet high in fat and sugar for 10 weeks, plus 8 weeks of HF diet + methyl group donor supplementation (HFMS n=8). Insulin Tolerance test was performed before sacrifice. Liver, epididymal and visceral fat, and serum samples were collected. Biochemical and histological analyzes were performed. In the liver, global DNA methylation was quantified and the transcriptome was analyzed using dual-channel microarrays. Proteomic analysis was carried out by immunoblotting.

**Results:** The supplemented animals (HFMS) showed a decrease in body weight epididymal and visceral fat ($p<0.001$). The HFMS group showed reduced serum levels of triglycerides and glucose and increased insulin sensitivity. Histological analysis of livers from ND and HFMS animals did not show characteristic MAFLD damage. Global DNA methylation was increased in the HFMS animals. Transcriptome analysis in the HFMS group showed a decrease in metabolic pathways associated with the development of MAFLD and an increase in lipid and cholesterol metabolic pathways. The proteomic analysis revealed an increase of the expression of H3K9 and DNMT1 and a decrease of H3K4, MJD2B and EZH1 proteins involved in the development of the disease.

**Conclusions:** Supplementation with methyl group donors has beneficial effects on weight and body composition, improves hepatic metabolism of lipids, and increases the expression of molecules that regulate DNA methylation and histones, even when consumption of a high-fat diet is continued.

### Ethical statement

The protocol was registered and approved by the Ethics Committee.

### Declaration of interests

None

### Funding

None

**Table 1**
Differences between diet groups

| | ND | HF | HFMS |
|---|---|---|---|
| **Final weight (gr)** | $30.25 \pm 2.25^{**}$ | $46 \pm 4.39^{**,\#}$ | $33.83 \pm 2.92^{\#}$ |
| **Feed consumption (gr)** | $3.417 \pm 0.37$ | $3.37 \pm 0.53$ | $3.164 \pm 0.36$ |
| **Liver weight (gr)** | $1.918 \pm 2.27^{*}$ | $2.3 \pm 0.36^{*}$ | $2.12 \pm 0.15$ |
| **Visceral fat weight (gr)** | $1.027 \pm 0.12^{*}$ | $1.433 \pm 0.39^{*,\#}$ | $0.98 \pm 0.19^{\#}$ |
| **Epididymal fat weight (gr)** | $1.58 \pm 0.31^{***}$ | $3.4 \pm 0.85^{***,\#}$ | $2.24 \pm 0.41^{\#}$ |
| **Glucose (mg/dL)** | $116.6 \pm 10.93^{*}$ | $137.13 \pm 19.19^{*,\#}$ | $132.9 \pm 13.3^{\#}$ |
| **Global Methylation (%)** | $0.6033 \pm 0.061^{*}$ | $0.5200 \pm 0.062^{*,\#\#}$ | $0.8967 \pm 0.17^{\#\#}$ |
| **Triglycerides (mg/dL)** | $82.67 \pm 5.508^{*}$ | $104.4 \pm 6.841^{*,\#\#}$ | $81.2 \pm 6.017^{\#\#}$ |

All the results are expressed as the mean ± SD. Statical analysis were performed using One-Way Anova and Tukey Post hoc test. The "#" symbol represents (#p<0.05) (##p<0.01) differences between HF vs. HFMS and "*" is used for HF vs. ND differences (*p<0.05) (**p<0.01) (***p<0.001).

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## Methyl-group donor supplementation beneficial effects on metabolic, histological, and inflammatory parameters in a murine model of alcoholic steatohepatitis

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**Introduction and Objectives:** Chronic alcohol consumption is the main cause of alcohol-related liver disease (ARLD) ranging a spectrum characterized by inflammation and progressive fibrosis. Currently, abstinence is the main treatment for ARLD; for this reason, it becomes indispensable to evaluate therapeutic alternatives as methyl group donors which have the potential to influence the development and progression of the disease. To evaluate the effect of chronic alcohol consumption coupled with methyl-group donor supplementation on metabolic and histologic features and gene expression of proinflammatory cytokines in a murine model of ARLD.

**Materials and Patients:** : 24 male C57BLC/6J mice divided into groups with conventional diet (ND n=8); alcohol-induced liver-injury induced with *ad libitum* consumption of a 20% ethanol-aqueous drink and a 45%-fat diet (OH n=8) for 18 weeks; or latter diet for 10 weeks, plus 8 weeks of this diet and methyl group (OH +METMIX n=8). This protocol is in the process of being approved by the CUCS Ethics, Research and Biosafety Committees.

**Results:** Serum biochemical studies and histological analysis performed in the methyl-donor supplemented group (OH+ METMIX) -zinc sulfate, methionine, vitamin B12, folic acid, betaine and choline- showed a significant decrease in body weight, epididymal and visceral fat ($p<0.05$), and serum levels of cholesterol, HDL and LDL. Whilst, serum levels of AST, ALT, TG and VLDL, as well as IL-6 and TNF-$\alpha$ mRNA from hepatic tissue, and the hormones insulin, leptin, glucagon, and resistance, demonstrated a tendency to decrease their concentrations compared to the OH group.

**Conclusions:** Treatment with methyl-group donors improves body weight, body composition, cholesterol, LDL and HDL concentrations, exerting beneficial and protective effects even when consuming an ethanol-aqueous drink.

### Ethical statement

The protocol was registered and approved by the Ethics Committee.

### Declaration of interests

None

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## Passenger lymphocyte syndrome, an unusual cause of anemia after liver transplantation

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**Introduction and Objectives:** The prevalence of anemia after liver transplantation ranges from 4.3% to 28.2%. Causes that occur in the first two weeks include bleeding, sepsis, medications, and hemolysis. Immune hemolysis represents less than 1% of the cases and includes graft-versus-host disease and hemolysis associated with ABO incompatibility. We present a case of passenger lymphocyte syndrome as a cause of immune hemolytic anemia two weeks after a liver transplant.